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TITLE: Novel Resuscitation from Lethal Hemorrhage Suspended Animation for Delayed Resuscitation

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14. **ABSTRACT** We have worked on novel ways to resuscitate combat casualties with exsanguination cardiac arrest (ExCA). We developed "suspended animation (SA)" using a hypothermic normal saline (NS) flush into the aorta after rapid (5 min) ExCA, in dog models. Using a NS flush we achieved intact recovery after ExCA of up to 2h at 7-10°C. SA has evolved into Emergency Preservation and Resuscitation (EPR). This is an ADDENDUM to the yr 6 report. In that report, we showed that EPR was effective even when ExCA was preceded by ~2h of hemorrhagic shock mimicking delayed evacuation. In yr 6, we also developed a rat EPR model and advised industries and tested prototypes for devices to bring EPR to the field. In this ADDENDUM, we report that in yr 7 we carried out tasks to optimize EPR and bring it to a clinical trial. 2h of EPR may be inadequate for some victims, thus we sought to extend its duration. Adding energy substrates to the NS flush, allowed us to achieve good outcome after 3h of EPR in dogs. We also studied neuronal death in our rat model and neuronal culture. A role for cardiolipin oxidation as a death trigger was shown. We held a meeting of trauma surgeons to plan a clinical trial of EPR for civilian ExCA. Our work led, in yr 7, to multiple publications, one patent, a feature in US News and World Report, the SCCM young investigator award, and presentations at TATRC day and ATACCC 2005.

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Table of Contents

BAA 99-1 PROPOSAL FOR YEAR 6

A. ABSTRACT

NOVEL RESUSCITATION FROM LETHAL HEMORRHAGE
Suspended Animation (SA) for Delayed Resuscitation
Keywords: Hemorrhagic shock, cardiopulmonary arrest, trauma, hypothermia, resuscitation, ischemia, proteomics, reperfusion, delayed neuronal death, combat casualty, terrorism, transport, emergency, preservation

We have been working since the 1980s, for the past 7 yrs under DOD support, on novel ways to resuscitate "unresuscitable" trauma victims. We focus on combat casualties who exsanguinate resulting within a few min in cardiac arrest (CA). We conceived and documented the concept of "suspended animation (SA)" using a hypothermic normal saline (NS) flush into the aorta after rapid (5 min) exsanguination (Ex) CA, in a clinically relevant dog outcome models. Using a NS flush we achieved intact recovery after ExCA of up to 2h at 7-10°C. SA has now evolved into concept Emergency Preservation and Resuscitation, defined by the acronym EPR. This is an ADDENDUM to the yr 6 (final) report. In that report, we indicated that EPR was effective even when ExCA was preceded by a prolonged period (~2 h) of severe hemorrhagic shock mimicking the case where a casualty is pinned down before evacuation--further supporting feasibility of EPR particularly for military use. In yr 6, we also developed a rat EPR model including miniaturized cardiopulmonary bypass and advised industries and tested prototypes for smart catheter insertion and cooling devices needed to bring EPR to the field. In this ADDENDUM, we report that in yr 7 we carried out additional tasks to optimize EPR and bring it to a clinical trial. Recognizing that 2 h of EPR may be inadequate for some trauma victims, we tested strategies to extend its duration. Adding energy substrates (dissolved oxygen and glucose) to the NS flush, allowed us to achieve good outcome after 3 h of EPR in dogs—a remarkable finding. In yr 7, we also continued studying neuronal death in both our rat EPR model and neuronal culture using proteomics and lipidomics. Reperfusion was shown to be critical to degradation of the rat brain proteome after prolonged ischemia. In addition, a role for cardiolipin oxidation in mitochondria as a death trigger was shown—suggesting a novel biomarker for apoptosis and new therapeutic opportunities. In June 2005, Dr. Samuel Tisherman held, in Pittsburgh, the first meeting of a consortium of trauma surgeons to plan a clinical trial of EPR for civilians with ExCA. Our work led, in yr 7, to multiple publications, one patent, a feature in US News and World Report, recognition by the SCCM with the young investigator award for trainee Dr. Xianren Wu, and presentation by Dr. Patrick Kochanek at the opening of TATRC day, the 2005 ATACCC meeting, and the keynote lecture to the Neurocritical Care Society.

NOTHING ON THIS PAGE IS PROPRIETARY INFORMATION

PI: Patrick Kochanek, MD

ANNUAL RESEARCH REPORT FOR USAMRMC/TATRC
October 1, 2004 – September 30, 2005

NOVEL RESUSCITATION FROM LETHAL HEMORRHAGE
Suspended Animation (SA) for Delayed Resuscitation
Project Year 6—ADDENDUM to Final Report

I. Introduction

This research report for 2004/05 is an **ADDENDUM** to our final report for our US Army funded research project on "Novel resuscitation from severe hemorrhage, suspended animation (SA) for delayed resuscitation" (PI: Dr. Kochanek, Co-PI: Dr. Tisherman). The final report (work through yr 6) was submitted and approved October, 2004. The work carried out from Sept 15, 2004 until Sept 30, 2005 represented **yr 7** of funding and addressed a number of tasks that we believe have continued to serve the goals of optimizing this new approach and brining it to a clinical trial. With a clinical trial in preparation (see below and body of this report), we were advised by the University of Pittsburgh Medical Center IRB, to develop a more scientific term than SA for our proposed hypothermic preservation strategy. Thus, we developed the acronym Emergency Preservation and Resuscitation (EPR). EPR, which we feel to also be a fitting homage to the late Dr. Peter Safar (the father of CPR), will be used to replace the term SA from this point forward in the proposal.

Recognizing that 2 h of EPR may be inadequate in some situations, such as where a casualty is pinned down or in cases where prolonged transport is necessary, we tested two strategies to try to extend the period of successful EPR to 3 h rather than 2 h. One of these two strategies—adding energy substrates (namely, dissolved oxygen and glucose) to the cold flush (i.e., "cold energy" flush)—allowed us to achieve good neurological outcome after EPR periods in dogs of 3 h. A second approach, using a novel ice slurry to cool, was not successful. In yr 7, we also continued study of the mechanisms of neuronal death in prolonged EPR in our rat model and in neuronal culture using proteomic and lipidomic approaches. A key role for reperfusion in degradation of the rat brain proteome was shown. Also, novel insight into the role of cardiolipin oxidation in mitochondria as a key trigger to neuronal death was shown. In June of 2005, Dr. Tisherman, held in Pittsburgh the first meeting of a consortium of trauma surgeons who will potentially serve as clinical investigators to bring this novel approach, to the preservation and delayed resuscitation of otherwise lethally injured civilians presenting with ExCA, to a clinical trial. Our work led, in yr 7, to multiple publications and presentations including recognition with the prestigious young investigator award from the Society of Critical Care Medicine for fellowship trainee Dr. Xianren Wu, and presentation by Dr. Kochanek of our findings as one of the opening plenary talks at the 2005 ATACCC meeting—along with a number of other prestigious presentations of this work at keynote and plenary sessions by both Drs. Tisherman and Kochanek. In addition, one patent is pending related to the cold energy strategy and a kit for proposed clinical use of EPR. Details of the findings from our work during yr 7 are presented in this ADDENDUM. Finally, despite continuing to accomplish a great deal, achieving the goals of 1) optimizing EPR in the experimental setting, and 2) bringing it to a clinical trial are, as one might imagine, a daunting task, that demands continued rigorous laboratory investigation, key

linkage with industry, and careful planning for a clinical trial. We plan to continue, in yr 8, to use remaining funds to achieve these two important goals.

II. Publication of work completed in yr 6

During yr 7, we published several manuscripts of work that was completed during yr 6. This included two manuscripts by Dr. Ala Nozari in the journal *Critical Care Medicine* (20) and the *Journal of Trauma* (21), and one by Dr. Xianren Wu in the *Journal of Trauma* (31). The paper by Dr. Nozari in *Critical Care Medicine* (20) garnered a very favorable editorial. Details of the findings in these three papers were outlined in prior reports and the references are listed in the publication list in this ADDENDUM.

III. Outcome studies in dogs

A. Strategies to extend the period of successful EPR from 2 to 3 h

1. "Cold Energy" flush

Recognizing that 2 h of EPR may be inadequate in some situations, such as where prolonged transport of the casualty is necessary, we tested two strategies to try to extend the period of successful EPR to 3 rather than 2 h. For induction of deep or profound hypothermia in EPR, we have been using an ice-cold normal saline (NS) solution. Solutions other than NS bear theoretical advantages in preserving vital tissues. However, the long-term outcome in terms of neurological function was not improved with albumin or Unisol (Behringer et al, 2004), nor with the University of Wisconsin solution in our model (Tisherman et al, 1991). Taylor et al (1995) reported that continuous perfusion with an asanguinous solution allowed satisfactory recovery over 3 h of CA, but it is not clear if the solution has brain-specific preservation potential in the absence of continued flow (Wu et al, 2005). A careful review of the literature suggested to us that during the induction phase of deep or profound hypothermia and even after target temperature is reached, the brain may continue to have substantial metabolic demands (~19% of base line at 18°C, or 11% at 8°C)(Ehrlich et al, 2002). This suggested to us two possibilities 1) re-charging the energy depleted brain with energy substrates added to the flush solution during the flush period–rather than solely attempting to lock the organism/brain in a state of preservation, and 2) providing some type of energy substrate(s) for consumption during the state of deep or profound hypothermic preservation. Of these two possibilities, the former is theoretically more likely to be successful, since the degree of energy depletion in the brain is likely to be considerable at normothermic arrest times beyond 2-3 min, and since the amount of an energy substrate that could be delivered during the induction of cooling is substantial since our typical flush rates approximate normal cerebral blood flow, and that cooling to a target temperature of 8°C takes ~12 min. Logically, we speculated that providing energy substrates during induction of EPR may either restore energy reserve or prevent further energy depletion and/or hypoxic/ischemic injury during the flush. During the period of established profound hypothermia in EPR, this approach may provide additional energy for consumption. Since other investigators have shown that intermittent flush of energy substrates into the brain during profound hypothermic cardiac arrest delayed depletion of ATP and creatine phosphate in the brain (Robbins et al, 1990), and the heart, we tested the hypothesis that a "cold energy" flush would allow a 3 h period of CA to be achieved.

Experimental design

The model included 3 phases: 1) a hemorrhage and CA phase (5+2 min); 2) an EPR phase (3 h), and 3) a delayed resuscitation phase, including CPB (2 h) and ICU Care (72 h). At the end of Hemorrhage and CA phase, dogs were randomized into 4 groups (Table 1):

Anesthesia and preparation

Custom-bred, male coonhound dogs, weighing 19.5-24.0 kg, were used. A total of 24 dogs were use and all dogs successfully completed the protocol. Dogs were fasted with free access to water for 12 h. Ketamine 10 mg/kg and atropine 0.4 mg was given IM. Following anesthesia induction with 4 % halothane via face mask, endotracheal intubation was performed. Continuous anesthesia was provided with halothane ~1%, titrated during preparation (O_2:N_2O: 50%:50%). Controlled ventilation was initiated with tidal volume 15-20 ml/kg, PEEP 2 cm H_2O, and frequency 20-25/min, titrated to maintain $PaCO_2$ 35-45 torr. EKG lead II was continually monitored. A cannula (18 G) was inserted into a peripheral vein and fluid infusion (D5W/0.45 NaCl at 4 ml/kg/h) was started. A Foley catheter was placed into the urinary bladder. Temperature probes were inserted for measuring rectal, esophageal, and both tympanic membrane temperatures (Tty). Sterile cutdowns were made in both groins and the right side of the neck. A PE 90 catheter was inserted into the left femoral artery for blood pressure monitoring and blood samples. A pulmonary artery catheter (7.5F) was inserted via the left femoral vein into the pulmonary artery to monitor pressure, cardiac output, and core temperature (Tpa). A CPB arterial cannula (7 or 9 G) was inserted into the right femoral artery. A multiple-hole cannula (17-19F) was inserted 25 cm into the right external jugular vein. The CPB system consisted of a hollow-fiber mambrane oxygenator (Medtronic, Grand Rapids, MI) and centrifugal pump (Biomedicus, Eden Prairie, MN). For induction of EPR, the CPB system was primed with flush solution; for delayed resuscitation after EPR, the system was primed with shed blood (30 ml/kg) and Plasma-Lyte A (Baxter, Deerfield, IL). Baseline measurements (hemodynamics, arterial and venous blood gases, body temperature) were collected when and the animal was stable, usually 15-30 min after surgical preparation.

Table 1. Experimental groups for "Cold Energy" flush

	Flush composition	O_2/N_2 insufflation
Group O_2+G+ (n=6)	**2.5% glucose in NS**	**O_2**
Group O_2+G-(n=6)	**NS**	**O_2**
Group O_2-G+ (n=6)	**2.5% glucose in NS**	**N_2**
Group O_2-G- (n=6)	**NS**	**N_2**

Hemorrhage, CA, and EPR phases

After two baseline measurements, heating, IV fluids, and halothane were discontinued, and the dogs were weaned to spontaneous breathing of air via a T-tube. When the canthal reflex returned, hemorrhage was initiated via the jugular venous cannulae and the blood was collected in bags with citrate anticoagulant for later reinfusion. Stepwise hemorrhage was controlled to reach mean arterial pressure (MAP) 20 mm Hg at 4 min. At 5 min, ventricular fibrillation was induced to ensure zero blood flow with transthoracic AC at 95 V. VF was confirmed with EKG. At 2 min after the onset of CA, flush solution (80 ml/kg) at 2°C was flushed into the aorta at a rate of 80 ml/kg/min with CPB (Biomedicus centrifugal pump). The close-chest CPB from the right external jugular vein to the right femoral artery was then initiated for induction of hypothermia until Tty reached 8C. Either 100% O2 or N2 was supplied to the oxygenator

throughout the EPR induction phase. The gas rate to the CPB oxygenator was adjusted to ensure a normal PCO_2 35-45 mmHg per blood gas analysis results. The whole body was covered with ice from the onset of flush to the end of 3 h CA.

Delayed resuscitation phase

At 3 h after occurrence of CA, reperfusion was started with cardiopulmonary bypass (CPB) that was primed with shed blood and heparin 1000 units. Just before the start of CPB, sodium bicarbonate (1 mEq/kg) and epinephrine 0.01 mg/kg were injected into the circuit. The temperature of the water bath of the CPB heat exchanger was set to 5°C > Tty/Tpa until Tpa reached 34°C. CPB was started with a flow of 50 $mL \cdot kg^{-1} \cdot min^{-1}$ when Tpa <20°C, increased to 75 $mL \cdot kg^{-1} \cdot min^{-1}$ when Tpa 21°C–30°C, and 100 $mL \cdot kg^{-1} \cdot min^{-1}$ when Tpa >30°C. Reinfusion of all shed blood was titrated to a CVP of 10–15 mm Hg. Repetitive doses of epinephrine (0.01 mg/kg) were given intra-arterially to increase MAP to 60 mm Hg when Tpa <20°C, to 80 mm Hg when Tpa 21–30°C, and to 100 mm Hg when Tty >30°C. When Tpa reached 32°C, defibrillation was attempted with external DC countershocks of 150 J, increased by 50 J each time for repeated shocks. Gas flow through the oxygenator was adjusted to keep $Paco_2$ at 30–35 mm Hg and Pao_2 >=100 mm Hg. During 2 hrs of CPB, controlled ventilation was with 100% oxygen at a rate of 8-10 cycles/min. The IV fluids were restarted at 4 mL/kg/hr. A base deficit of >6.0 mEq/L was corrected with sodium bicarbonate. MAP was controlled at 90–150 mm Hg. The CPB flow rate for assisted circulation was reduced to 75 and 50 $mL \cdot kg^{-1} \cdot min^{-1}$ and stopped at 120 mins. During CPB, activated clotting times were maintained at >300 secs with heparin.

The details of life support, including mechanical ventilation, hemodynamic monitoring and support, and correction of acid-base or electrolyte abnormalities, were published previously. ICU care, including mechanical ventilation, was provided for 48 h. The body temperature was kept at 34°C until 36 h of delayed resuscitation, followed by slow rewarming (0.3°C/h). At 48 h, anesthetics were discontinued and muscle relaxant reversed. Dogs were then weaned from mechanical ventilation. After extubation, they were transferred to a stepdown unit where continuous IV fluids and vital sign monitoring were provided until 72 h.

Functional and neuropathological outcome

Functional outcomes were evaluated every 6 h according to overall performance categories (OPC) (OPC 1 = normal or slight disability; 2 = moderate disability; 3 = severe disability; 4 = coma; and 5 = death) and neurologic deficit scores (NDS) (NDS 0 - 10% = normal; 100% = brain death), which includes level of consciousness, breathing pattern, cranial nerve function, sensory and motor function, and behavior. Blood samples were obtained at baseline and every 24 h for cardiac (troponin I, CPK MB fraction) and liver enzymes (transaminases and bilirubin). At 72 h, a final functional assessment was performed and dogs were then re-anesthetized with ketamine and halothane. Perfusion-fixation was performed with cephalad infusion of 10% neutral buffered formalin via the thoracic aorta. A gross necropsy was subsequently performed. The brain was removed ~2 h after perfusion fixation and retained in 10% neutral buffered formalin until the time of dissection. Whole perfusion-fixed brains were divided into multiple coronal slices. Six coronal brain slices plus three transverse sections of the medulla oblongata and upper cervical cord were selected for microscopic evaluation. These represented whole brain slices taken at the following levels: 1) the optic chiasm; 2) the anterior thalamus; 3) the posterior thalamus; 4) the midbrain; 5) the occipital lobes; 6) the middle of the cerebellum and

underlying brainstem; 7) the medulla oblongata and upper cervical cord. Each brain slice was divided into between two and four pieces so that the sections would fit onto standard 1 x 3 inch microscope slides (excepting for the sections through the medulla oblongata and cervical cord). These brain slices were processed for paraffin embedding, resulting in 20 tissue blocks from each brain. The blocks were sectioned at 5 micrometers and the sections stained with H&E and with Fluoro-Jade B. The examining neuropathologist (RG) was blinded to treatment. Each neuroanatomic region with evidence of damage on microscopic examination received a grade from 1+ (minimal) to 5+ (severe). Affected regions on each side of the brain (right and left) received separate scores in H&E-stained and Fluoro-Jade B-stained sections. The Histological Damage Scores (HDS) were determined by adding up the individual scores (for each region).

Data are presented as mean ± SD unless otherwise stated. Repeated measures ANOVA was performed followed by Bonferroni post-hoc tests to identify differences in physiological parameters between groups. NDS and HDS scores were analyzed using Mann-Whitney U Test, and Fisher's exact test was used to assess differences in OPC proportions (i.e., good outcome [OPC 1] vs bad outcome [OPC 2-5]) between groups. A *p*-value <0.05 was considered statistically significant.

Table 2. Physiological parameters at the end of SA induction

Groups	Group O^+G+	Group O^+G-	Group O^-G^+	Group O^-G^-
Flush duration (min)	26.2±1.5	26.0±2.3	24.9±2.2	27.1±2.3
Glucose(mg/dl)	975±186	175±60	957±295	288±15
PO2 (mmHg)	432->740	410->740	23.5±7.8	18.6±8.1
pHa	7.2±0.1	7.1±0.0	7.1±0.0	7.1±0.1
PCO2 (mmHg)	34.4±3.8	41.6±3.4	35.3±4.4	39.9±4.1
BE (mmol/L)	13.8±3.0	-13.1±3.5	-15.5±1.0	-15.2±1.9
Lactate (mmol/L)	6.4±1.0	6.7±1.7	7.6±1.0	8.8±0.8
Hematocrat (%)	11.3±2.8	15.5±2.8	9.5±4.3	14.7±3.5
Sodium (mmol/L)	132.9±7.1	147.3±2.3	136.6±0.8	145.9±1.4
Potassium (mmol/L)	3.5±0.3	3.1±0.2	4.8±0.6	5.0±0.6

Results

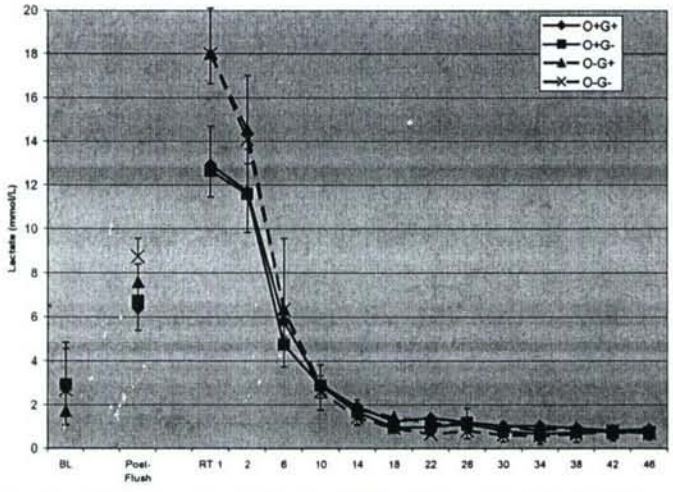

Fig 1. Arterial lactate level during EPR of dogs for 3 h. Addition of oxygen to the flush (either with or without glucose) resulted in lower lactate levels ($p<0.05$) vs either group without oxygen.

The total flush time to reach Tty 8°C was similar between groups. Two G+ groups had lower sodium levels ($p<0.05$), numerically lower perfusion pressures (NS), and hematocrit (NS) at the end of flush (Table 2). Two O+ groups had lower potassium than the other two groups ($p<0.01$) (Table 2). The lactate levels were higher in the two O- groups during early delayed resuscitation phase ($p<0.05$) (Fig. 1). At 72 h, all dogs in the O+G+ group regained consciousness with a better NDS (Fig. 2) and better OPC (Table 3) (both $p<0.05$), vs the O- groups. In the Group O+G-, only

4 dogs regained consciousness (NS vs other groups). All but 1 dog in the O- groups remained comatose. HDS of the O+G+ group in the neocortex appeared to be the best, while HDS of the O-G+ group was the poorest in most brain regions ($p<0.05$ vs other groups, please see Fig. 3 for representative neuropathology).

Finally, in separate pilot experiments, EPR was induced in a small series of dogs with and without the addition of oxygen and glucose supplementation. Brain tissue measurements of ATP and related purine degradation products were measured in the laboratory of collaborator Dr. Edwin Jackson in University of Pittsburgh Center for Clinical Pharmacology. Preliminary data suggested variability in the findings, which we believe may be related to technical issues in rapid sampling of brain tissue and the labile nature of ATP. We will continue to pursue this approach in an attempt to document direct effects of the energy substrates on markers of tissue energetics. Alternatively, we cannot rule out other effects including effects during reperfusion and further study is needed.

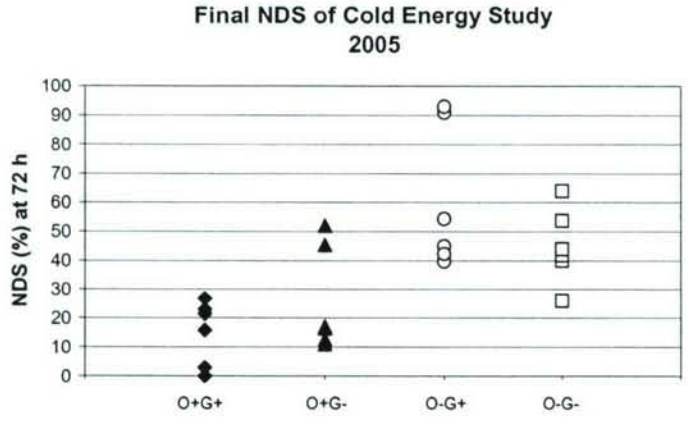

Figure 2. Final NDS after 3 h of EPR and 72 h of recovery. Dogs with oxygen added to the flush solution showed the best outcomes and the O+G+ group had better outcome than either of the groups without oxygen.

Discussion

The most important finding in this study was that 3 h of EPR (over 2.5 h of no-flow) can be reliably reversed to achieve a conscious outcome of either normal or moderate disability. This is a remarkable finding and with our study design appears to be highly dependent on the addition of oxygen to the flush and also dependent, at least in part, on the addition of glucose. The ability to achieve 3 h of EPR, and over 2.5 h of a deep hypothermic circulatory arrest, is a novel finding of considerable importance to both the potential applicability of EPR and toward directing the future investigation for this program. This study opens a new avenue in the pursuit of EPR, the addition of energy substrates and oxygen carriers to the cold flush. It also supports the notion that once in the state of EPR, if technically possible, intermittent periods of saline perfusion with energy substrates (even dissolved oxygen alone) could potentially extend the duration that can produce a favorable outcome. It is also important, however, to recognize that despite intact functional outcomes by OPC and NDS (established outcome tools modified that have been used in modified form in human clinical trials), neuropathologic examination using contemporary methods including Flurojade B staining

Table 3. Final OPC at 72 h after EPR for 3 h duration.

	Group O+ G+	Group O+ G-	Group O- G+	Group O- G-
5 Dead				
4 Coma		*	***	**
3 Severe disability		*	***	***
2 Moderate disability	****	**		*
1 Normal	**	**		

OPC=Overall performance category; G=Glucose, O=oxygen;
*Each symbol represents an individual dog studied

reveal that some subtle neuronal injury remains at these prolong ischemia durations. This was the case also with the use of a conventional saline flush (without energy substrates) for a 2 h period of EPR. Thus, we believe that it is vital to test the "cold energy" strategy in the 2 h EPR paradigm to determine if completely intact functional and histopathological outcome can be achieved (i.e., better than with NS alone), and those studies are proposed as a further addendum to this work. Similarly, additional adjuncts to be added to the flush should be tested. For example, it would be extremely valuable to test a hemoglobin carrier such as polynitroxylated hemoglobin to determine if this further enhanced the efficacy of the cold energy strategy. Those studies are proposed as an important part of a white paper that was submitted to congress for consideration within the 2006 Department of Defense appropriation and entitled "Applied Emergency Hypothermia for Advanced Combat Casualty Care." The addition of energy substrates to the flush might allow the use of higher temperatures that 8°C to achieve a good outcome—particularly at EPR durations of <90 min. This could greatly facilitate the feasibility of EPR (by reducing flush volumes), particularly for field use.

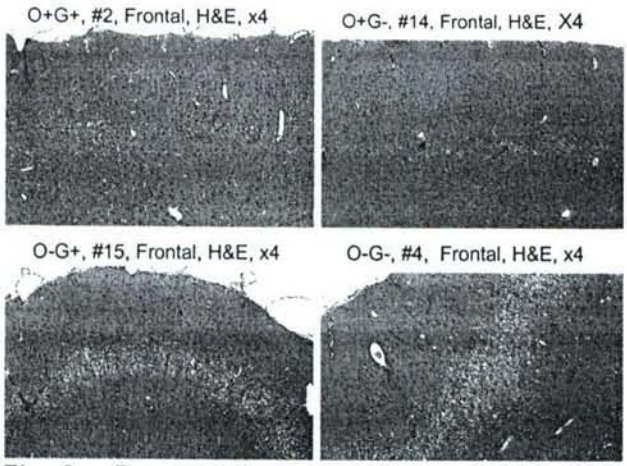

Fig. 3. Representative neuropathology (H&E-stained sections) from frontal lobe in dogs in the 3 h EPR study. Neuroprotection is evident in frontal lobe sections stained with H&E in Oxygen+ Glucose + (O+G+) and O+G- treated dogs (top panels). In contrast, obvious laminar necrosis is seen in O-G+ and O-G- treated dogs (bottom panels).

Finally, we also noted that addition of glucose without oxygen is highly deleterious during EPR. This is consistent with a wealth of data in cerebral ischemia research and clinical practice showing deleterious consequences of elevated blood glucose concentration during brain ischemia. This is believed to be due to increased lactate production. In contrast, when oxygen was added to the flush and lactate levels were the lowest, the addition of glucose as an energy substrate was beneficial in our model. Publications and presentations from the entire project, along with those specifically during this ADDENDUM period are listed in the Publications section. Specifically related to this research on the "cold energy" flush strategy, we will present a paper at the 2005 meeting of the International Anesthesia Research Society (see abstract 28 in "Publications" section). In addition, one patent is pending on the use of the cold energy flush for EPR induction along with an EPR kit.

2. Ice slurry flush

A second strategy was tested, in two pilot experiments, to attempt to limit the time required to achieve target temperature during the induction of EPR in dogs. In collaboration with Dr. Lance Becker and his group of scientists at the University of Chicago and Argonne National Laboratories, we infused, into the large-bore femoral catheter, an ice-slurry preparation that they have developed and which has been shown to be efficacious in a number of settings to rapidly induce hypothermia. Theoretically, the amount of heat consumed in melting the microcrystals in

the slurry to a liquid is substantial, and should dramatically facilitate cooling rate. Our hypothesis was that this ice-slurry flush would facilitate extremely rapid cooling to target temperature of 8°C or less. Unfortunately, although the use of an ice-slurry has promise in a number of settings for the induction of hypothermia, for EPR, the rapid infusion of this slurry to re-fill the circulation after exsanguination CA resulted in crystallization or solidification of the slurry in the abdominal aorta. This produced partial obstruction with extremely high flush pressures and precluded the homogenous delivery of flush to the entire organism. Cooling of the lower half of the body (as reflected by rectal temperature) was extremely prompt, but brain (tympanic) temperature fell very gradually. After two pilot studies, it was deemed that this approach would require considerable additional modification or re-design if it were to be tested again, and was tabled—particularly in light of the success of the cold energy flush strategy. Of note, Dr. Becker and his team of scientists from Argonne visited the center to both plan and directly collaborate in these experiments.

IV. Studies of mechanisms of neuronal death in prolonged EPR

A. Rat model of EPR

The dog model has been used to maximize clinical relevance, including full ICU care and long-term outcome assessment. However, certain limitations are pertinent to that model. First, there are few molecular tools available for dogs, limiting the study of cellular and molecular mechanism of secondary damage/repair. Understanding molecular mechanism after ischemia-reperfusion in EPR would allow us to assess markers of reversibility and define specific molecular targets for future therapy. Second, the cost and labor-intensiveness of the experiment in dogs pose an important obstacle to rapid screening of promising drugs to provide additional brain and extracerebral tissue preservation (either alone or in combination with hypothermia). As described in the final report, we developed a rat model of EPR to begin to address the aforementioned limitations. This included the development of CPB in rats in our center—since CPB is essential to successful resuscitation from the profound or deep hypothermia in EPR. In the final report last year we described a 30 min rat EPR for the initial trial. We indicated that an abstract on this work had been submitted to the annual meeting of the Society of Critical Care Medicine. That abstract was presented by fellow Dr. Tomas Drabek, who received one of the poster awards at the meeting for his presentation (see abstract #20 in "Publications" section).

During the period of the ADDENDUM, we carried out studies to decrease the amount of flush required for EPR in rats. We assessed survival and neurological outcome as primary parameters and markers of organ injury as secondary outcome parameters. We also included a CPB-only control group. Three groups were studied: (1) hypothermic EPR (H-EPR, 0°C flush with Plasma-Lyte A, n=6); (2) normothermic EPR (N-EPR, 38°C flush, n=6); (3) control group (60 min of CPB at 38°C, n=6). For the H-EPR group, target temperature 15°C was achieved with 275 ml of flush. The same flush volume was used for the N-EPR group. EPR duration was 20 min. After 20 min of H-EPR or N-EPR, reperfusion was started with CPB primed with collected shed blood, and mechanical ventilation was restarted. All rats in H-EPR and control groups survived, while none of the rats in N-EPR group had restored cardiac activity or survived. All rats in the H-EPR and control groups achieved OPC 1 from Day 2. NDS was also normal or near normal in all rats in H-EPR and control groups and did not differ between them. Brains from N-EPR group were

characterized by edema, and some had early evidence of neuron necrosis. Brains from the H-EPR group were normal in appearance, although minimal degrees of Fluoro-Jade staining were present in a minority of rats. No lesions were present in the brains of rats in the control group. Enzymatic markers of organ injury (ALT, AST, CPK, CPK-MB, urea) were normal in H-EPR and control rats on d 7. These studies showed that a 20 min EPR can be achieved in rats with a limited flush volume and excellent outcomes. This second report on EPR in rats was presented by Dr. Drabek at the 2005 Shock Society meeting and was accepted for presentation at the Annual meeting of the American Society of Anesthesiologists (see abstracts #26 and #27 in the "Publications" section). A full manuscript is in preparation (see #8 in the "Publications in preparation" section). This study sets the stage for treatment trials of novel agents that are being proposed for 2006.

In the projected experiments using this rat EPR model, as described above, we will test drugs that show promise to augment EPR. We also plan to add neurobehavioral studies to evaluate learning and memory skills in long-term survivors, using Morris water maze methodology. Also, we are examining molecular mechanisms that come into play during EPR, especially markers and mediators of a cell-death pathways—using proteomics and lipidomics (see below). Finally, we also plan to extend the duration of EPR that is achievable in the rat model.

B. Proteomic/degradomics assessment of protein degradation in EPR in rats

We have examined global hippocampal proteins changes after complete global cerebral ischemia (CGCI) as occurs during CA using multiple proteomic approaches to further examine the neuroprotective actions of hypothermia. Prior studies using a large format 2D gel approach showed surprisingly minimal differences in high copy protein degradation or change when comparing 30 min of CGCI without reperfusion at either 38 or 10°. However, significant decreases in both pyruvate dehydrogenase (PDH) and eukaryotic initiation factor 2 beta (eIF2B) were found during hypothermic vs normothermic CGCI without reperfusion--implicating changes in the two rate controlling proteins for oxidative metabolism and protein synthesis. Those findings were described in the final report. Over the last year (the period of this addendum report) we sought to further evaluate global protein degradation during CGCI with and without hypothermia using more powerful solublization buffers and 1D gel analysis. Using a decapitation complete ischemia model in Sprague-Dawley rats (n=6/group), both hippocampi were rapidly dissected and randomized to 30 min of complete ischemia at either 38or 10°C. A third group of hippocampi (no ischemia) served as controls. We separated proteins from hippocampal lysates (paired samples in triplicate) using medium format (16x18 cm) SDS-PAGE. Proteins were stained with Sypro Ruby, imaged, and quantified. No global differences in protein levels were found among either normothermic or hypothermic ischemia groups or controls without reperfusion (one-sample sign test) suggesting little degradation had occurred.

Based on these data we then began studies of 30 min of complete ischemia followed by 60 min of reperfusion using the rodent CPB model comparing normothermic to hypothermic rats. Degradomics is the study of global protein degradation during in injury or disease. Prior studies from our laboratory using a large format 2D gel proteomic approach showed surprisingly minimal difference in protein degradation when comparing changes after 30 min global cerebral ischemia at either 38 or 10 °C (please see yr 6 Final Report). However, weaker solublization

buffers are required for 2D vs traditional SDS 1D gel analysis. We sought 1) to further evaluate global protein degradation during complete brain ischemia with and without hypothermia using more powerful solublization buffers and 1D gel analysis with an extremely sensitive protein dye (Sypro Ruby - 1 ng detection sensitivity), and 2) to assess the effect of reperfusion. Two models were used for these studies. First, a decapitation complete ischemia model was used in Sprague-Dawley rats (n=6/group). After isoflurane anesthesia and decapitation, both hippocampi were rapidly dissected and randomized to 30 min of CGCI at either 10 or 38°Ç, then frozen at –70°Ç for subsequent protein analysis. A third group of rapidly dissected hippocampi (no ischemia) served as controls. To study the effect of reperfusion after prolonged (30 min) CA, at either 10 or 38°C, we used our new rat CPB model with CA followed by reperfusion via miniaturized CPB. Separation of proteins by molecular weight from hippocampal lysates was accomplished with medium format (16x18 cm) vertical SDS-PAGE. Paired samples were run in triplicate on the same gel to reduce variability, stained with Sypro Ruby, imaged and quantified. Using non-parametric statistical analysis no differences in protein levels were found between either hypothermic or normothermic ischemia groups or controls without reperfusion. In contrast, reperfusion after 30 min of normothermic but not hypothermia resulted in marked protein degradation. We observed little degradation in the rat hippocampal proteome during prolonged normothermic and hypothermic CGCI. Reperfusion appears to be critical to protein degradation after prolonged ischemia in rats. Our data are consistent with studies by Powell et al (2005) after cardiac complete ischemia with reperfusion suggesting that protein oxidation and ubiquitination may be responsible for this enhanced global protein degradation seen after complete ischemia with reperfusion but not after complete ischemia without reperfusion. We will further explore this possibility in the upcoming year by examining these post-translational protein modifications after reperfusion.

However, we realized that the study of mostly high copy proteins with either 2-D or the 1-D global approach above is limiting and have turned to an additional proteomic approach using high-throughput immunoblotting. First, we examined low copy proteins using high throughout immunoblotting with 387 brain specific antibodies (BD laboratory Powerlblot) on the same brains samples in normothermic or hypothermic 30 min CGCI without reperfusion to examine more specific proteins with greater sensitivity. This study showed many more protein changes (~25% of the 387 low copy proteins examined) than were seen with either 2-D or 1-D gel analysis of high copy global proteins. Importantly, this study showed a marked effect of hypothermia on the downregulation of important protein kinases such as PKC gamma and on vesicular transport proteins important in neurotransmitter release and reuptake--both consistent with the anti-excitotoxic role of hypothermia treatment. In addition, proteins important in protein ubiquitination and degradation were differentially modulated by hypothermia suggesting that even during the ischemic insult, modification of the ubiquitin degradation system occurs with hypothermia treatment. Over the next year we will examine this same protein array with high-throughput immunblotting in rats receiving CGCI with reperfusion. We are excited about the utility of this proteomic method to reveal new information regarding global low copy protein changes during and after normothermic and hypothermic CGCI and anticipate much new information will be forthcoming over this next year. Finally, Dr. Mandeep Chadha, a fellow working on this project, presented an abstract at the 2004 SCCM congress during the ADDENDUM period (see abstract #23)

C. Studies of oxidative lipidomics in neuronal culture

Programmed cell death is triggered by acute brain injury yet its reliable detection is complicated by effective clearance of apoptotic cells *in vivo*. During the period of last funding period our consultants (Kagan and Bayir) have described a new biomarker of early apoptosis in the brain: oxidized cardiolipin (CL). These studies were carried out as an initial application of lipidomics by our investigative group. Our ultimate goal is to apply these methods to our rat EPR model to serve both as a method to detect apoptotic neurons and help define novel targets for antioxidant therapies—ie., oxidation of CL within mitochondria.

Primary cortical neurons were treated with the non-oxidant pro-apoptotic agent, staurosporine (STS) 1 μM, for 4h and the amount of phospholipid hydroperoxides were determined. Briefly, following STS treatment, lipids were extracted and separated by two-dimensional thin layer chromatography (2D-HPTLC). Spots corresponding to different phospholipids were scraped from the plates, treated with phospholipase A2 and incubated with microperoxidase-11 in the presence of Amplex Red. This protocol allows for quantitative analysis of lipid hydroperoxides based on stoichiometric production of resorufin that can be sensitively and reliably determined by fluorescence HPLC. The content of phospholipid hydroperoxides can be compared with the abundance of a particular phospholipid class. Treatment of primary cortical neurons with STS resulted in decreased cell viability assessed by the conversion of 3-[4,5-dimethylthiazol]-2,5-diphenyltetrazolium bromide to formazan, cyt c release assessed by ELISA in mitochondrial and cytosolic fractions, caspase-3 activation glutathione depletion and phosphotidyl serine externalization assessed by flow cytometry. Mitochondrial electron transport activity was determined by measuring Succinate oxidase activity. Peroxidase activity of cytochrome-C (cyt-c) was determined by a chemiluminescence assay. ESI-mass spectra of CL isolated from cortical brain mitochondria was acquired on a triple-quadrupole tandem mass spectrometer (Finnigan MAT TSQ 700, San Jose,CA) equipped with an electrospray interface.

Using above techniques we have shown that treatment of primary cortical neurons STS resulted in apoptosis manifested by cyt c release, caspase-3 activation, glutathione depletion and PS externalization. Using oxidative lipidomics, we established that robust and selective CL oxidation occurred as early as 1h after STS exposure and preceded appearance and accumulation of all the other apoptotic biomarkers. In mouse brain mitochondria, removal of 85% -90% of cyt c by alamethacin treatment caused complete inhibition of succinate oxidase activity but did not affect peroxidase activity. Addition of exogenous cyt c resulted in reconstitution of succinate oxidase activity but did not change the peroxidase activity. In the presence of exogenously added CL, reconstitution of succinate oxidase activity was incomplete and dependent on the CL/cyt c ratio. Thus cyt c in brain mitochondria can act as a peroxidase likely activated during apoptosis. Our ESI/MS analysis showed that molecular species of brain CL include polyunsaturated long chain fatty acid residues such as C22:5 and C22:6, highly susceptible to oxidation. We found that CL was the only phospholipid that underwent oxidation while more abundant brain phopholipids (PC and PE) remained nonoxidized after brain trauma. This selective and robust (7-fold) CL oxidation was detectable as early as 3h after trauma. Thus, early accumulation of CL hydroperoxides can be used as a biomarker of apoptosis that is not masked by effective clearance of apoptotic cells in the brain. It also suggests that CL is a key potential target of oxidative stress early in the cascade of neuronal death. Potent antioxidants capable of penetrating neurons and/or

mitochondria may be a critical therapy. These findings are also consistent with our prior report showing that the brain penetrating antioxidant Tempol improved functional outcome in our EPR dog model (Behringer et al, 2002).

This work done in neuronal culture has been submitted as an abstract to 2006 Annual Society of Toxicology Meeting (see Abstract #29). As described above, we believe that these studies have provided important mechanistic insight into a key neuronal death pathway (apoptosis), and we hope to be able to translate this work to the rat EPR model and possibly the dog outcome model in future studies.

V. Clinical consortium to develop a protocol for initial use of EPR in civilian trauma resuscitation of exsanguinations CA

A. Consortium Meeting

On the day following the Safar Symposium and lecture, Drs. Tisherman and Kochanek hosted the first meeting of the clinical consortium of trauma centers to plan a clinical feasibility trial for EPR. Representatives from the Maryland Shock Trauma Center, Oregon Health and Science University, University of Texas-Houston, Massachusetts General Hospital, and Allegheny General Hospital, as well as, the Department of Defense were present. The group reviewed the laboratory experience with EPR from both the Safar Center and the Uniform Services University (Drs. Rhee and Alam). All were in agreement that a feasibility trial is warranted. The group then discussed potential inclusion and exclusion criteria for enrollment in such a study. The most appropriate subjects may be patients with either blunt or penetrating trauma who exsanguinate to cardiac arrest either in the emergency department or shortly before arrival. Standard resuscitation, including aggressive fluid resuscitation and an emergency department, should be initiated. If the patient remains pulseless after brief resuscitative efforts, EPR could be initiated by placing an arterial cannula into either the aorta or the left ventricle and a venous cannula into the right atrium. The idea of direct left ventricle cannulation, suggested by Dr. Rodriguez, seemed like an excellent approach to rapid access (it merits testing in our dog model, and this important study will be carried out to 72 h outcome with remaining funds this year). Ice-cold NS would then be infused and recirculated (when possible) to achieve tympanic membrane temperature of 10°C. The patient could then be transported to the operating room for resuscitative surgery. The goal is to include 10-20 subjects.

Prior to initiation of this study, key team members at each center will need to be trained in EPR techniques. The training may involve cadavers and/or animal experiments, either at the Safar Center or at the centers. We are currently seeking support for this through the aforementioned white paper that was submitted to congress for consideration within the 2006 Department of Defense appropriation and entitled "Applied Emergency Hypothermia for Advanced Combat Casualty Care," along with other funding mechanisms.

Representatives from the University of Pittsburgh IRB joined the group near the end of the day at the consortium. There was general agreement that this type of study was appropriate and met the criteria for the emergency exception from informed consent. As discussed in the introduction, the IRB recommended the elimination of the term SA.

The group will plan to review the minutes from this meeting and begin regular teleconferences to further develop the protocol for the trial and begin training of personnel.

VI. Other accomplishments of the EPR program during the period of this ADDENDUM report

Devices developments: Dr. Lyn Yaffe continues to serve as an important consultant and coordinator, linking the industrial groups interfacing with this project. He facilitates linkage to industry in the development of methods and devices for EPR. Specifically, during this funding period, he held a weekly conference call with the Safar Center and industrial partners, and played an important role in drafting the patent on "cold energy" flush and the use of a kit to induce EPR (formerly EPR). Industrial partners include Lyn Yaffe (smart catheter project), Kathy Saxman and David McMurray, Ardiem (portable cooler project), Carleton Hisa and Li Ma, Synzyme Technologies (nitroxide-based resuscitation strategies). Dr. Yaffe also visits Pittsburgh about once per month. The project of Dr. Yaffe includes Dr. Klain, and as advisors, Drs. Kochanek and Tisherman, and Mr. S. W. Stezoski. These conference calls have proven invaluable to our team.

Xianren Wu, MD Served as senior fellow on the dog EPR project in 2005 under the mentorship of Drs. Kochanek and Tisherman. He has remarkable experience, particularly for a fellow, in the field of HS including work in dog, pig, and rat models. His productivity regarding publications has been exceptional. During this ADDENDUM period, He received the Young Investigator Award from the SCCM for his work on the successful use of EPR in the setting of prolonged hemorrhagic shock (see Abstract #21). That award is the highest honor given to a trainee by the SCCM. That paper was also presented at the annual fellow's day sponsored by the AHA PA-Delaware Affiliate in January, 2005 (see abstract #17). The full manuscript of that work is now in submission at the journal *Circulation*. During this ADDENDUM period, Dr. Wu also carried out, as research team leader, the remarkable study showing that the addition of oxygen and glucose could facilitate successful outcome from 3 h of EPR. That work was submitted to the IARS (International Anesthesiology Research Society) for presentation this year (see abstract #28). The full manuscript of that important work is in preparation. Of note, Dr. Wu was accepted into the anesthesiology residency of the University of Pittsburgh School of Medicine and began clinical training in July of 2005. During the Addendum period Dr. Wu was the author or co-author on 3 peer-reviewed manuscripts (#s 20,21 and 31), two reviews (#24, #25), one chapter (#29), and editorial (#30), and a remarkable 9 abstracts (#s 19-22,24-28)

Mandeep Chadha, MD a fellow working under the direction of Drs. Jenkins and Kochanek used proteomics to study protein degradation and modification during complete global cerebral ischemia with profound hypothermia. Dr. Chadha completed his Critical Care Medicine training and was funded by a T-32 entitled Pediatric Neurointensive Care and Resuscitation Research from the NICHD/NIH on which Dr. Kochanek is PI and Dr. Jenkins is a trainer. This project on proteomics in EPR has broad implications across the field of resuscitation and is an outstanding opportunity for fellowship training. Dr. Chadha presented numerous abstracts of his novel work in proteomics and an initial full manuscript is in preparation (Abstract #s9,12,13,18,23). During the ADDENDUM period he presented an abstract (#23) as previously described above. Finally,

Dr. Chadha began on the Faculty of the University of Texas Southwestern as an Assistant Professor at Children's Hospital of Dallas in fall of 2005.

Tomas Drabek, MD is now a senior fellow in charge of development of the rat EPR model. He will also serve as team leader for the dog EPR studies in the upcoming year. He was a cardiac anesthesiologist in Prague, Czech Republic, and his experience in that regard is perfect to expand the relevance of our rat EPR model to the study of DHCA in cardiac surgery. During the ADDENDUM period, he presented three abstracts (#s 20,26 and 27) of his rat EPR work and co-authored a chapter during yr 7 (see manuscript #29). The first manuscript on the EPR model in rats is in preparation.

Dr. Robert Garman: During yr 7, we added Dr. Robert Garman to our group as a consultant. Dr. Garman is an internationally recognized neuropathologist who has added state-of-the-art neuropathologic assessment of both the dog and rat brain outcomes to our work.

Dr. Hülya Bayır: During yr 7, we added Dr. Hulya Bayir as a consultant on this project. She is a promising young clinician-scientist in the department of critical care medicine with an impressive track record of study in the area of oxidative stress. She is working in collaboration with the talented group of Dr. Valerian Kagan in the University of Pittsburgh Center for Free Radical and Antioxidant Health.

During yr 7 (the period covered in this ADDENDUM report), our group gave an impressive 22 presentations on the EPR project (please see abstracts and also the section below on "Lectures."). This included abstracts presented by Drs. Wu, Drabek, and Chadha, and invited lectures by Drs. Kochanek and Tisherman. In addition, we published 3 manuscripts, multiple chapters (see publications), and have one patent pending on this work.

Third Annual Safar Symposium at the University of Pittsburgh School of Medicine: On June 23, 2004, the T*hird Annual Safar Symposium* was held at the University of Pittsburgh School of Medicine. This event was attended by ~150 clinicians and scientists. The morning session focused on "Breakthroughs in Resuscitation" and focused on the inflammatory response to brain injury and resuscitation. The afternoon focused on the use of simulation in resuscitation research. The symposium featured prominent national and international speakers and was supported in part by this grant. The program is attached in the appendix. We again thank the US Army for supporting this symposium in honor of the late Dr. Safar.

Books, monographs, and Lectures: Dr. Tisherman's book on hypothermia in acute medicine entitled 'Therapeutic Hypothermia" was published by Kluwer. It included 253 pages and, germane to this project, a chapter on the use of EPR in exsanguination CA. During the year, Dr. Kochanek gave two important lectures on this EPR project for the US Army including a keynote lecture at TATRC day and one of the two opening plenary lectures at the ATACCC meeting. In addition, he presented this work as the keynote speaker at the 2005 meeting of the Neurocritical Care Society, and in plenary format at the Annual International Pediatric Cardiac Intensive Care Conference, the International Society of Critical Care in Brussels, the University of Maryland Dept. of Anesthesiology, at McGill University and Montreal Children's Hospital, and as the first Thomas Vargo Professor at the Texas Children's Hospital in the Baylor College of Medicine.

Similarly, Dr. Tisherman presented this work at the Annual Trauma Conference in Las Vegas hosted by Maryland Shock Trauma.

Media Coverage: Our work on this project was featured by a remarkable number of top media sources including US News and World Report (April 29, 2005), the Wall Street Journal (April 20, 2005), The Pittsburgh Post Gazette (April 21, 2005), the Pittsburgh Tribune Review (June 29, 2005), and a news segment on WTAE-television in Pittsburgh (June 28, 2005). The video segment can be viewed on the Safar Center website (www.safar.pitt.edu). Copies are available on request.

VII. Key research accomplishments (during the period of this ADDENDUM)

A. We showed that EPR (formerly SA) with profound hypothermia can be importantly enhanced by adding the energy substrates to the flush, namely, dissolved oxygen and glucose. Specifically, a remarkable 3 h period of CA at profound hypothermia can be tolerated with intact functional outcome in dogs using this approach. This has important implications for potential clinical use in the transport of injured victims during EPR for exsanguination CA. This approach (adding energy substrates to the flush) could also have the potential to minimize or eliminate subtle neuropathological injury at shorter EPR durations than 3 h.

B1: We have used a newly developed rat model of EPR with reperfusion using miniaturized CPB to demonstrate that reperfusion is critical to degradation of the hippocampal proteome in rat brain.

B2: Our consultants (Drs. Kagan and Bayır) have demonstrated that cardiolipin oxidation is a potential marker of neuronal death by apoptosis and is a likely mitochondrial target for antioxidant therapies.

C: We have assembled a consortium of clinical investigators to begin to draft a protocol and define the population for an initial trial of EPR in civilian trauma resulting in exsanguination CA.

VIII. Reportable outcomes

Specific reportable outcomes for the ADDENDUM funding period (yr 7) and are defined in the report and are identified with an asterisk **(*) and bolding** in the reference list—including publications and presentations and patents.

IX. Conclusions

Work in yr 7 as outlined in this ADDENDUM report has accomplished the remarkable achievement of 3 h of EPR in dogs. If these findings translate to humans, it suggests that the use of a "cold energy" flush has the potential to facilitate transport of casualties even if prolonged periods are needed for transport such as 2-3 h. This finding could have important implications for cardiac or neurological surgery or cerebral protection—whenever deep or profound hypothermia are needed. Our work in a new rat model of EPR supports a key role for reperfusion in mediating degradation of the proteome in brain and our studies in neuronal culture suggest an important new target for oxidative stress in brain—namely, cardiolipin in mitochondria. Supported by this grant, we also established a clinical consortium of trauma clinical-investigators for a clinical trial of EPR in civilian trauma. Finally, in future studies, we

will further refine EPR in our dog and rat models while moving toward implementation of a clinical trial.

X. Appendices

This appendix list includes all items generated by the EPR project during yrs 6-7. **(Please note that publications and other items generated during the period of this ADDENDUM are identified with an asterisk (*) and bolding).** Reprints of these specific items from yr 7 are attached in the appendix (available upon request).

XI. Publications

1. Tisherman SA, Rodriguez A, Safar P: Therapeutic hypothermia in traumatology. Chapter in Surgery Clinics of North America 79:1269-1289, 1999.

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12. Safar PJ, Tisherman SA: Trauma resuscitation: what have we learned in the last 50 years? (Editorial review) Curr Opin Anaesthesiol 16:133-138, 2003.

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15. Kochanek PM, Safar PJ: Therapeutic hypothermia for severe traumatic brain injury. Invited editorial, JAMA 289:3007-3009, 2003.

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18. Tisherman SA. Suspended animation for resuscitation for exsanguinating hemorrhage. Crit Care Med 32:Suppl 2, S46-50, 2004.

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Books and Monographs

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Articles in submission

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Publications in preparation

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Patent and Trademark

United States Provisional Patent:
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Inventors: PM Kochanek, SA Tisherman, X Wu, SW Stezoski, LJ Yaffe

Publications in the lay press related to this project:

***April 20, 2005 The Wallstreet Journal: Suspended Animation**
***April 21, 2005 The Pittsburgh Post-Gazette: Suspended Animation**
***May 23, 2005 US News and World Report: Hibernation**
***June 28, 2005 WTAE-TV interview: Suspended Animation**
***June 29, 2005 Pittsburgh Tribune Review: Pitt Scientists Resurrect Hope of Cheating Death**

Lectures relevant to this project by Patrick Kochanek, MD

1. Hunting for Breakthroughs in Resuscitation and Neurointensive Care. American Heart Association, Pittsburgh, Pennsylvania, February 13, 2004.

2. Breakthroughs in resuscitation research. Grand Rounds, National Center for Child Health and Development, Tokyo, Japan, February 6, 2004

3. Novel Resuscitation from Lethal Hemorrhage –Suspended Animation for Delayed Resuscitation. Telemedicine and Advanced Technology Research Center (TATRC) Product Line Review, Fort Detrick, Maryland, July 13, 2004.

4. A Novel Approach to Cerebral Resuscitation: Suspended Animation using Profound Hypothermia for Delayed Resuscitation. Hypothermia in Clinical Practice, Houston, Texas, September 11, 2004.

***5. A Novel Approach to Cerebral Resuscitation: Suspended Animation with Delayed Resuscitation. Keynote address. 3rd Annual Meeting of the Neurocritical Care Society, Scottsdale, Arizona, February 26, 2005.**

***6. A Novel Approach: Emergency Hypothermia with Delayed CPR. International Symposium on Intensive Care and Emergency Medicine, Brussels, Belgium, March 21-25, 2005.**

***7. Head Injury in Children: Present Guidelines. International Symposium on Intensive Care and Emergency Medicine, Brussels, Belgium, March 21-25, 2005.**

***8. Traumatic Brain Injury. International Symposium on Intensive Care and Emergency Medicine, Brussels, Belgium, March 21-25, 2005.**

***9. A Novel Approach to Cerebral Resuscitation: Suspended Animation with Delayed Resuscitation. Department of Anesthesiology Neuroprotection Seminar Series, University of Maryland, April 5, 2005.**

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***12. Cellular and Molecular Mechanisms of Secondary Injury in Pediatric TBI. Pediatric Academic Society and Society for Pediatric Research Annual Meeting, Washington, DC, May 14-17, 2005.**

***13. Serious CNS Injury in the PICU Population. Grand Rounds, University of Texas, Department of Pediatrics, Houston, Texas, May 27, 2005.**

***14. New Developments in Brain Research at the Safar Center. Research Conference, University of Texas, Department of Pediatrics, Houston, Texas, May 27, 2005.**

***15. CPCCRN, the PCCR Program: The relationship with NCMRR and NICHD. Program and Growth in NCMRR, Rockville, MD, June 21, 2005.**

***16. Emergency Hypothermia. ATACCC 2005 Conference, St. Pete Beach, FL, August 15-17, 2005.**

Lectures relevant to this project by Samuel Tisherman, MD

Add new lectures

SCRR 2003-2004 Annual Report
(Please see enclosure)

Mild hypothermia during prolonged cardiopulmonary cerebral resuscitation increases conscious survival in dogs*

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Objective: **Therapeutic hypothermia during cardiac arrest and after restoration of spontaneous circulation enables intact survival after prolonged cardiopulmonary cerebral resuscitation (CPCR). The effect of cooling during CPCR is not known. We hypothesized that mild to moderate hypothermia during CPCR would increase the rate of neurologically intact survival after prolonged cardiac arrest in dogs.**

Design: **Randomized, controlled study using a clinically relevant cardiac arrest outcome model in dogs.**

Setting: **University research laboratory.**

Subjects: **Twenty-seven custom-bred hunting dogs (19–29 kg; three were excluded from outcome evaluation).**

Interventions: **Dogs were subjected to cardiac arrest no-flow of 3 mins, followed by 7 mins of basic life support and 10 mins of simulated unsuccessful advanced life support attempts. Another 20 mins of advanced life support continued with four treatments: In control group 1 (n = 7), CPCR was with normothermia; in group 2 (n = 6, 1 of 7 excluded), with moderate hypothermia via venovenous extracorporeal shunt cooling to tympanic temperature 27°C; in group 3 (n = 6, 2 of 8 excluded), the same as group 2 but with mild hypothermia, that is, tympanic temperature 34°C; and in group 4 (n = 5), with normothermic venovenous shunt. After 40 mins of ventricular fibrillation, reperfusion was with cardiopulmonary bypass for 4 hrs, including defibrillation to achieve spontaneous circulation. All dogs were maintained at mild hypothermia (tympanic temperature 34°C) to 12 hrs. Intensive care was to 96 hrs.**

Measurements and Main Results: **Overall performance categories and neurologic deficit scores were assessed from 24 to 96 hrs. Regional and total brain histologic damage scores and extracerebral organ damage were assessed at 96 hrs.**

In normothermic groups 1 and 4, all 12 dogs achieved spontaneous circulation but remained comatose and (except one) died within 58 hrs with multiple organ failure. In hypothermia groups 2 and 3, all 12 dogs survived to 96 hrs without gross extracerebral organ damage ($p < .0001$). In group 2, all but one dog achieved overall performance category 1 (normal); four of six dogs had no neurologic deficit and normal brain histology. In group 3, all dogs achieved good functional outcome with normal or near-normal brain histology. Myocardial damage scores were worse in the normothermic groups compared with both hypothermic groups ($p < .01$).

Conclusion: **Mild or moderate hypothermia during prolonged CPCR in dogs preserves viability of extracerebral organs and improves outcome. (Crit Care Med 2004; 32:2110–2116)**

Key Words: cardiac arrest; resuscitation; hypothermia; extracorporeal circulation; survival; neurologic deficit; dog

Sudden cardiac death remains the principal killer in industrialized countries (1, 2). The potential physiologic potency of standard external cardiopulmonary-cerebral resuscitation (CPCR) far exceeds the current rates of achieving conscious survival after out-of-hospital CPCR attempts. In about 50% of cases, restoration of spontaneous circulation (ROSC; i.e., spontaneous heartbeat) is not achieved in the field and resuscitation efforts are abandoned (1–3). Among patients who reach the hospital intensive care unit, about one half die in the intensive care unit, primarily from cardiac, cerebral, or multiple organ failure (1–3). Among long-term survivors, 10–30% have permanent brain damage. Rapidly induced mild hypothermia after ROSC from prolonged normothermic ventricular fibrillation (VF) cardiac arrest (CA; i.e., no flow) has improved cerebral outcome in dogs (4–8) and patients (9–11). For cases resistant to ROSC attempts, we searched for a method to preserve the organism during transport to, and preparation for, prolonged circulatory support using cardiopulmonary bypass (CPB), which would allow the heart to recover from ischemic stunning or be evaluated, repaired, or replaced (12–16).

For CPCR-resistant cases of CA, we considered "suspended animation for delayed resuscitation" during no-flow, with tympanic temperature (Tty) of 10°C, which is being explored primarily for preservation of the exsanguinating trauma victim to allow transport and resuscitative surgery during pulselessness (17–19). In clinical cases, however, with hearts resistant to ROSC attempts (perhaps only temporarily), clinicians would hesitate to create no-flow deliberately with aortic cold flush, instead of continuing basic (BLS) and advanced life support (ALS) with steps A (airway), B (breathing), and C (circulation by chest com-

*See also p. 2164.

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pressions) with the hope that the heart may resume beating. Other ways to "buy time" might include mild (33–36°C) or moderate (27–32°C) hypothermia during steps A–B–C until CPB is initiated. Others and we have used these definitions for temperature levels of therapeutic hypothermia.

We hypothesized that a) maintaining viability of brain, heart, and organism during prolonged CPCR basic and advanced life support steps A–B–C (low flow) can be a bridge during transport to initiation of CPB in the hospital; b) induction of mild hypothermia during CPCR can maintain viability for ≥40 mins of VF-CA; and c) mild hypothermia (which is safe) is as effective as moderate hypothermia (which has more risk of complications) in preserving the viability of the heart during CPCR.

MATERIALS AND METHODS

The experimental protocol was approved by the Institutional Animal Care and Use Committee of the University of Pittsburgh. The care and handling of the animals followed guidelines of the National Institutes of Health. All surgery was performed by the same team in our animal intensive care unit, using sterile techniques (20–21).

Protocol. The model's protocol, with life support to 96 hrs after reperfusion, simulated a scenario of prolonged VF resistant to defibrillation attempts using CPCR steps A–B–C over 40 mins to "bridge" from collapse via transport to initiation of CPB in the hospital emergency department. Twenty-seven custom-bred hunting dogs (19–29 kg body weight, age 8–12 months) were used. They were sedated with ketamine 10 mg/kg intramuscularly. Anesthesia was induced with halothane 2–4% in N_2O/oxygen 50/50% via a cone mask. The dogs were then positioned supine, intubated, and mechanically ventilated to maintain an arterial P_{CO_2} at 35–40 mm Hg. A positive end-expiratory pressure of 5 cm H_2O was applied. Anesthesia was maintained during preparation with halothane 0.5–1.5% and N_2O/oxygen 50/50% without neuromuscular blockade. Temperature probes were inserted for measuring tympanic membrane (Tty), esophageal, and rectal temperatures. Tty was controlled at 37.5 ± 0.1°C with heating blankets and heating lamps before the insult. We chose to control Tty since the brain was the primary target organ for our therapy. We recognize that the correlation between Tty and brain temperature is poor, although one recent study suggested good correlation between Tty and brain temperature in neurosurgical patients (22). The correlation between core and brain temperatures is also variable (23). Gastric and bladder catheters were inserted. Dextrose 5% in sodium chloride 0.45% was administered at 5 mL/kg/hr via a peripheral intravenous cannula (18 gauge). A 10-Fr catheter was inserted into the left femoral artery to monitor arterial pressure and for blood sampling. A 7- to 8-gauge cannula was inserted 3 cm into the right femoral artery for later use for CPB. A pulmonary artery catheter (7.5 Fr) was inserted via the left femoral vein and advanced into occlusion position for pressure and temperature monitoring, continuous cardiac output determination, and blood sampling. Arterial and central venous pressures and electrocardiogram were continuously recorded on a polygraph. Due to technical issues, venous pressures were not measured accurately during chest compressions.

To control Tty in groups 2, 3 and 4, via venovenous extracorporeal shunt cooling, a 13-Fr catheter was inserted via the femoral vein 20 cm into the inferior vena cava and connected to 15 m long tubing (3 mm inner diameter; primed with isotonic saline 120 mL and 500 IU heparin) immersed in ice water. This simple system was used instead of conventional heat exchangers specifically because of its simplicity. Only one pump was needed. The system could readily be used outside of the hospital. There was no additional systemic heparinization. A shunt flow of 10 mL/kg/min by a roller pump returned the cooled blood via the right external jugular vein into the superior vena cava using a multiple-holed 19-Fr catheter.

Randomization for group assignments was performed after surgical preparation but before the insult began so that one team member could prepare materials for cooling if necessary. Attempts were made to keep the other team members blinded to group assignments until during the insult.

After stabilization and two baseline measurements, intravenous fluids were discontinued, heating devices were turned off, and the dogs were weaned to spontaneous breathing via a T-tube. VF was induced with a 95-V AC, 60-Hz transthoracic shock of 2 secs, using subcutaneous needles. The shock was repeated as needed. Pulselessness was allowed to persist for 3 mins before initiation of cardiopulmonary resuscitation (CPR). CPR basic life support (BLS) steps A–B–C, with air for ventilation to simulate bystander CPR, was then initiated, using left parasternal chest compressions (dogs turned 45° to the right of supine to exert more direct pressure on the heart) with a mechanical thumper (Michigan Instruments, Grand Rapids, MI), rate of 80/min. The depth of compression was titrated to maximize systolic blood pressure. There was no active decompression of the chest. Tidal volumes of approximately 15 mL/kg (larger than the standard in humans based on greater compliance of the dogs' chest and lungs) at an F_{IO_2} of 0.21 were delivered with a self-inflating bag (Laerdal Medical, Stavanger, Norway) at a ratio of five compressions to one ventilation. In previous experiments with this model, this ratio provided better hemodynamics and ventilation during CPR than the 15:2 ratio. There was no interruption of compressions for ventilation. After 7 mins of BLS (10 mins of VF), to simulate arrival of paramedics and futile ROSC attempts, ALS was continued for another 10 mins of normothermic VF, using three external transthoracic DC countershocks of 50 J in rapid sequence. In pilot experiments, even countershocks of ≥150 J after 3 mins of untreated VF and 7 mins of BLS were unsuccessful. Deliberately weak shocks (50 J) were used in the study to avoid premature defibrillation, which would have made that experiment unusable. These shocks never caused defibrillation; VF persisted. Chest compressions were continued at a rate of 60/min (decreased per protocol to maximize systolic blood pressure based on previous experience with this model) and ventilation with F_{IO_2} 1.0, at a ratio of 5:1, was used. The primary goal was to maximize cerebral perfusion pressure. Epinephrine 20 μg/kg was administered intravenously at 5-min intervals from 10 mins VF to 20 mins, without any additional defibrillation attempts. The dogs were to be maintained in VF for a total of 40 mins. Lidocaine (1–1.5 mg/kg intravenously) was administered for recurrent or refractory ventricular fibrillation or ventricular tachycardia.

At VF 20 mins, the dogs were assigned to one of four treatment groups: Control group 1 (n = 7) received continued normothermic CPR-ALS until VF 40 mins, without venovenous shunt flow, with Tty maintained at 37.5°C using heating blankets as necessary. Moderate hypothermia group 2 (n = 7) received venovenous shunt cooling to Tty 27°C during 20 mins of CPR-ALS. Mild hypothermia group 3 (n = 8) was treated as group 2 but received cooling to Tty 34°C. Cooling in groups 2 and 3 was induced with a bolus of 20 mL/kg normal saline at 2°C into the superior vena cava, followed by venovenous extracorporeal pumping at 200 mL/min (estimated to be 10% of cardiac output). In normothermic group 4 (n = 5), the initiating intravenous flush (at 37°C) and venovenous pumping were similar to groups 2 and 3 but were maintained at normothermia. No additional epinephrine or countershocks were administered during this time.

Reperfusion after VF 40 mins was with CPB as an experimental tool since ROSC attempts with external CPCR would not be reliable after such a severe insult. The use of CPB for resuscitation also simulates a possible clinical scenario for cases of refractory VF. The CPB system used a centrifugal pump to circulate venous blood from the superior vena cava catheter into the femoral artery cannula via a membrane oxygenator (12–16). The CPB system had been primed with lactated Ringer's solution 400 mL with sodium bicarbonate 2 mEq/kg. CPB flow was maintained at 100 mL/kg/min. After 15 mins of recirculation with CPB, defibrillation attempts were initiated with external DC countershocks of 150 J

(which could now be successful since the heart has been reperfused), increased if needed by 50 J for repeated shocks. If ROSC was achieved (defined as the presence of arterial pulsations on the arterial pressure tracing), CPB was continued for assisted circulation. By protocol, Tty should be approximately 34°C at this time point. Epinephrine 5 μg/kg was administered intravenously before countershocks and then repeated as needed every 5 mins until ROSC. After ROSC, norepinephrine was titrated to maintain the mean arterial pressure at 90–120 mm Hg. Flow of 100% oxygen through the oxygenator was adjusted to keep $Paco_2$ at 30–35 mm Hg. The CPB flow rate was kept at 100 mL/kg/min until 120 mins and then reduced to 50 mL/kg/min until weaning from CPB at 4 hrs. Weaning was initiated earlier if the dogs achieved ROSC and were hemodynamically stable without inotropic support. The temperature of the water bath of the CPB heat exchanger was set to 34°C. After CPB, Tty 34°C was maintained by external means until 12 hrs in all four groups.

When CPB was initiated, controlled ventilation was resumed with 100% oxygen, which was maintained until weaning from CPB. The intravenous maintenance fluid was restarted. A base deficit of >6.0 mEq/L was treated with sodium bicarbonate intravenously.

Intensive care was continued until 96 hrs or earlier death by technicians and critical care physicians. Controlled ventilation was continued to ≥48 hrs. Neuromuscular blockade was maintained with intermittent doses of pancuronium (0.1 mg/kg intravenously). Analgesia was with N_2O/oxygen 50/50%. In addition, throughout the experiment of 96 hrs, intravenous boluses of morphine (0.1–0.3 mg/kg) and diazepam (0.1–0.3 mg/kg) were titrated to prevent signs of pain (reactive wide pupils or hypertension). Hypotension (mean arterial pressure <80 mm Hg) was treated with normalization of central venous pressure (5–8 mm Hg) and intravenous titration of norepinephrine. Standard intensive care included airway suctioning, periodic deep lung inflations, and position change (rotation). The dogs received cefazolin (250 mg intravenously) every 8 hrs for infection prophylaxis. At 44–48 hrs, neuromuscular block was reversed with neostigmine (50 μg/kg) plus atropine (25 μg/kg) and the dogs were weaned to spontaneous breathing via T-tube and extubated by previously reported criteria (13–20). Dogs that required continued circulatory support were ventilated for an additional 24 hrs. Successfully weaned dogs were transferred to a stepdown intensive care unit for observation and treatment to 96 hrs, with oxygen by mask, continuous monitoring of pulse rate, and arterial oxygen saturation. The maintenance fluid was dextrose 5% in NaCl 0.45% until 48 hrs and dextrose 10% in NaCl 0.45% thereafter, until the dog either could drink adequately, died, or completed the experiment after being killed at 96 hrs.

Outcome Evaluation. Performance was evaluated according to overall performance categories (OPC): 1, normal (able to walk and eat); 2, moderate disability (able to sit but not stand or walk); 3, severe disability (unaware of surroundings, withdraws to pain); 4, coma (some reflexes or pathologic movements, but no response to pain); and 5, death (13–16, 20). This scoring system is designed to be similar to the Glasgow Outcome Scale used clinically. Neurologic function was evaluated as neurologic deficit scores (NDS 0–10% = normal; 100% = brain death), which includes evaluation of consciousness, breathing, cranial nerves, sensory/motor function, and behavior (13–21). Our group, and others, have used these outcome measures >20 yrs for large animal outcome experiments. OPC and NDS were evaluated every 8 hrs after extubation for best (at any time) and final values (at 96 hrs). Attempts were made to discontinue any sedation ≥4 hrs before final evaluations at 96 hrs. If necessary, sedation was reversed with naloxone (1.5–6.0 μg/kg intravenously) and/or flumazenil (0.1 mg intravenously), repeated if needed. The 96-hr NDS evaluation was the average of four evaluators.

After final evaluation at 96 hrs, the dogs were reanesthetized for morphologic studies. Via a left thoracotomy, brain perfusion fixation, with infusion of paraformaldehyde into the aortic arch, for cutting, staining, and histologic damage scoring, was performed as described previously (17, 20, 21). The same six brain slices were stained with hematoxylin-eosin-phloxine. Using light microscopy, the same pathologist, blinded for treatment assignments, scored 19 distinct anatomical brain regions for severity and extent of ischemic neuronal changes, infarcts, and edema (21). A total histopathologic damage score (HDS) of >40 represents moderate damage, and >100 represents severe damage.

A complete necropsy was performed. Macroscopic lesions in the myocardium were scored as absent, minimal, mild, moderate, marked, or severe and were scored taking into account the pattern, appearance, and anatomical distribution (0 = no damage, 100 = severe damage). Although a score was given, this was a qualitative appraisal of overall myocardial damage, without a true quantitative scoring system.

Statistical Analysis. Repeated-measures analyses of variance were performed followed by Bonferroni-Dunn *post hoc* tests to identify differences in hemodynamic variables and temperature data between groups over time. NDS, HDS, and myocardial damage scores were analyzed using Mann-Whitney U test, with the sequentially rejective Bonferroni test being used to preserve the experiment-wise type-I error rate at 0.05. Fisher's exact test was used to assess differences in OPC proportions (dichotomized to OPC 1 and 2 = good outcome [similar to the Glasgow Outcome Scale with potential for independent functioning] and OPC 3, 4, or death = bad outcome) between groups. A $p < .05$ was considered statistically significant.

RESULTS

Three of the 27 dogs were excluded from analysis. One each in groups 2 and 3 had severe, exsanguinating hemorrhage from major liver lacerations secondary to chest compressions. These were clearly direct, severe liver injuries, not minor injuries complicated by coagulopathy. One in group 3 had subarachnoid hemorrhage and *Dirofilaria immitis* infection.

There were no significant differences between groups in baseline measurements, including hemodynamic variables and blood gas, electrolytes, serum glucose, hemoglobin, and hematocrit values.

Despite three countershocks of 50 J during ALS, all 24 dogs remained in VF until reperfusion was initiated with CPB and countershocks with >150 J were delivered. All dogs then achieved ROSC after 15–120 mins of CPB (Table 1). Temperatures (Fig. 1) and blood pressures (Fig. 2) changed as expected, according to protocol. Coronary perfusion pressures (diastolic arterial pressure-central venous pressure) after ROSC were initially slightly above baseline (Fig. 2) but returned to baseline values by 12 hrs. All 12 normothermic dogs (groups 1 and 4) died with cardiovascular and multiple organ failure during intensive care, with the exception of one dog in group 1 that survived to 96 hrs in coma (Fig. 3). Median survival time was 25 hrs (range, 4–96) in group 1 and 15 hrs (4–24) in group 4 (nonsignificant between these two groups). All 12 hypothermic dogs (groups 2 and 3) were successfully weaned from CPB and controlled ventilation and survived to 96 hrs ($p < .0001$ vs. normothermia groups 1 and 4) with normal or near-normal function and brain histology (Fig. 3). If the excluded animals were included (intention to treat analysis), the survival in the hypothermia groups would still be significantly greater than that in the normothermic groups ($p < .0001$).

During CPCR, mean arterial pressures were 39–63 mm Hg and mean diastolic pressures were 24–39 mm Hg without difference between groups (Fig. 2). In groups 2, 3, and 4, these pressures increased after the intravenous flush of normal saline at VF 20 mins and returned to preinfusion levels within 8 mins, with-

Table 1. Resuscitation variables

Group	Control	Tty 27°C	Tty 34°C	Tty 37.5°C
Countershocks, total no.	2 (1–19)	1	1 (1–16)	14 (1–25)
Countershocks, total energy, J	300 (150–3990)	150	150 (150–3200)	2550 (150–4000)
Time of ROSC, mins after start of CPB	22 (15–120)	15	17 (15–33)	32 (15–105)
Total bicarbonate, mEq	115 (50–215)	95 (50–175)	107 (50–130)	100 (95–275)
Total epinephrine, mg	0.9 (0.4–6.4)	0.5 (0.4–1.1)	0.8 (0.5–1.7)	0.9 (0.4–1.7)
Total norepinephrine, mg	6.68 (2.32–85.46)	2.05 (1.26–5.40)	7.49 (2.01–36.32)	23.09 (10.88–49.58)
Duration of NE infusion, mins	3.5 (1–38)	6 (0.25–44)	2.25 (0.5–80.25)	12 (4–24)
Lidocaine, mg	20 (0–280)	25 (20–184)	30 (20–40)	20 (20–30)
Survival time, hrs	25 (4–96)	96	96	15 (4–24)

Tty, tympanic temperature; ROSC, restoration of spontaneous circulation; CPB, cardiopulmonary bypass; NE, norepinephrine.
Data are given as median (range).

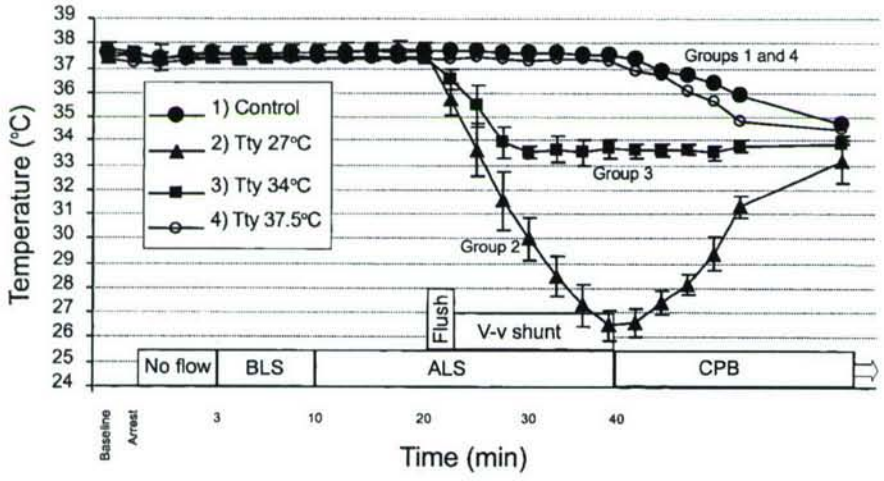

Figure 1. Tympanic temperature (*Tty*) during 3 mins of normovolemic ventricular fibrillation (no flow), followed by 7 mins of basic life support (*BLS*) and 30 mins of advanced life support (*ALS*). *CPB*, cardiopulmonary bypass; *V-v shunt*, venovenous shunt cooling. Data are presented as mean and SD.

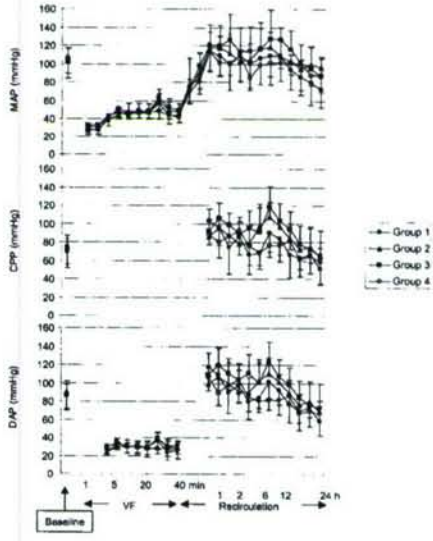

Figure 2. Mean (*MAP*) and diastolic arterial pressures (*DAP*) and coronary perfusion pressure (*CPP*) during and after ventricular fibrillation (*VF*) cardiac arrest. Data are presented as mean and SD.

out statistical differences between groups. After ROSC and after weaning from CPB, heart rate values were significantly higher in normothermic flush group 4 vs. hypothermic flush groups 2 and 3 ($p < .001$ and $p = .006$, respectively), despite adequate analgesia, normalization of central venous pressure, and avoidance of drugs with chronotropic effects. No other differences were observed in the hemodynamic variables between the groups. Mean arterial pressure was controlled per protocol. Cardiac output was not available in several dogs because of difficulty advancing the catheter. After weaning from CPB, cardiac output was quite variable within groups. Values were not statistically different from baseline, nor were they different between groups.

Arterial P_{CO_2} values were also variable. These values were frequently high (50–60 torr) at the time of initiation of CPB but returned to baseline levels per protocol by CPB 15 mins. P_{CO_2} ranged from 30 to 40 torr during CPB and early resuscitation. There were no differences between groups.

Venovenous shunt cooling in groups 2 and 3 was initiated by an intravenous saline flush, which decreased Tty from 37.5°C to 36.1 ± 0.7°C (Fig. 1). Pulmonary artery temperature was 26 ± 0.2°C and esophageal temperature 35.8 ± 1.5°C at the end of the intravenous flush. The time needed for shunt cooling after the flush to achieve Tty 34°C in groups 2 and 3 was only 2 mins (a decrease in Tty of 1°C/min). In group 2, continued venovenous shunt cooling decreased Tty to a nadir of 26.6 ± 0.6°C at the end of CPCR ALS (VF 40 mins). Within 15–20 mins after recirculation with CPB, these temperatures reached 34°C in all groups and were maintained at that level until 12 hrs.

Extracerebral organ failure, after CPB and ROSC, was the main reason for irreversible deterioration in normothermic groups 1 and 4. Arrhythmias appeared within the first hour after ROSC in all four groups. The most frequent form of arrhythmia was multifocal runs of ventricular extrasystoles, appearing intermittently in all dogs, especially during the first day after the insult. During CPB (assisted circulation), after ROSC, ventricular arrhythmias increased gradually in two dogs in each of the normothermia groups 1 and 4. Shortly after these four dogs were weaned from CPB, they died in VF that was resistant to vigorous CPR and up to 25 countershocks. Two dogs in normothermia group 1 and the remaining three dogs in normothermia group 4 died within 38 hrs after recirculation in vasopressor-resistant shock. Two other dogs in normothermic group 1 developed an increasing need for large doses of norepinephrine (despite adequate left ventricular filling pressures), severe metabolic

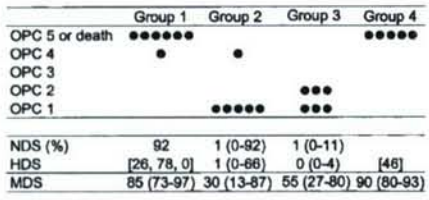

Figure 3. Final 96-hr outcome. Overall performance categories (*OPC*): OPC 1, normal; OPC 2, moderate disability; OPC 3, severe disability; OPC 4, coma; OPC 5, brain death. *NDS*, neurologic deficit score; *HDS*, total brain histologic damage score; *MDS*, gross myocardial damage score. Each dot represents a dog. Values of NDS, HDS, and MDS are expressed as median (range). Brackets represent individual HDS scores at 22–58 hrs of reperfusion.

acidemia, anuria, and respiratory failure. These dogs were killed at 25 and 38 hrs, respectively, when it was recognized that they were not salvageable, to obtain their brains for histologic scoring.

Myocardial injury was present in all four groups, despite patent coronary arteries. Milder lesions were restricted to the subendocardium and subepicardium with a patchy, multicentric pattern that coalesced into larger, focally extensive lesions. Superficial subendocardial hemorrhage and papillary muscle necrosis were more frequently observed in the left than in the right ventricle and more frequently in the normothermia groups than in the hypothermia groups. Microscopically, the areas of myocardial damage were characterized by the loss of fiber cross-striation, decreased nuclear definition, and prominent contraction band necrosis. Intensely eosinophilic transverse bands representing hypercontracted myofibrils span these degenerative cells. In some areas, there was frank associated individual myofiber coagulation necrosis with associated disintegration and loss of fiber integrity. Another finding seen in areas adjacent to frankly degenerative myocardium, especially in subendocardial and subepicardial perivascular locations, was focal myocytolysis (vacuolar degeneration). Some hearts also had large amounts of prominent basophilic stippling of myofibers.

The total myocardial damage scores were significantly lower in the hypothermia groups 2 and 3 than in the normothermic groups 1 and 4 ($p = .0083$) but did not differ between the two hypothermia groups ($p = .1548$, Fig. 3).

Cerebral outcome is illustrated in Figure 3. There was 100% agreement among observers regarding OPC scoring. The only dog in normothermic groups 1 and 4 that survived to 96 hrs remained comatose (OPC 4) and required controlled ventilation. In contrast, all surviving dogs in group 3 and all but one dog in group 2 had good functional outcome (OPC 1 or 2, Fig. 3). Because of early deaths, only four brains of the normothermic groups could be studied histologically: three in group 1 and one in group 4. Histologically, the brains in the normothermic groups were characterized by multifocal infarctions, with vasculitis/encephalitis adjacent to the infarcted areas. These lesions were noted in the frontal, parietal, and occipital cortexes as well as the putamen and caudate nucleus. Multifocal vasculitis was also observed adjacent to an infarcted area in the occipital cortex of one dog in hypothermia group 3. Histopathologic changes in groups 2 and 3 were mild and consisted mainly of isolated ischemic neurons and, in two dogs, focal vasculitis. The only region in which the regional HDS was not zero was the caudate nucleus. Except for these lesions, total brain HDS was normal (0–4%) in all but two dogs in group 2 and in all dogs in group 3 ($p < .001$ for hypothermia groups vs. normothermic groups).

DISCUSSION

This study strongly supports all three hypotheses posed in the introduction. The results show outcome benefit for simulated "refractory" CA cases, in terms of overall organ preservation, survival time, and survival rate, with mild (34°C) or moderate (27°C) hypothermia, induced by venovenous extracorporeal shunt cooling, during prolonged CPCR-ALS steps A–B–C, as a bridge to temporary, prolonged CPB. The benefit derived from mild hypothermia after ROSC for cerebral recovery has been well documented (4–11). All four groups in the present study with VF of 40 mins were treated with mild hypothermia after ROSC; this alone did not prevent organ failure and early death in groups 1 and 4. Demonstration of the added benefit (without negative side effects) of mild (group 3) or moderate (group 2) hypothermia, when introduced during CPR steps A–B–C (low flow), not only for the brain but also for preservation of extracerebral organs, is new. The finding that the impact of hypothermia was so pronounced on extracerebral organ preservation was not expected.

Mild hypothermia initiated before CA in rats was shown to be more beneficial than after CA (24). The results of the present study in a clinically realistic dog model document that therapeutic hypothermia should be initiated as soon as possible, even before ROSC, to provide effective protection from, or to mitigate, post-CA damage to all vital organs. Even though one of us recommended resuscitative moderate hypothermia as a step in the CPCR system as early as 1961 (25), it has not been practiced because of a fear of causing arrhythmias, depressing the myocardium, and causing coagulopathy and infection and because of practical limitations with slow surface cooling (26, 27). Moderate hypothermia (30°C) has been considered detrimental during CPCR, as it may worsen the chance for achieving ROSC, in addition to aggravating the myocardial damage (6, 28). The cardiovascular safety of even 27°C in this study in healthy animals may not apply to patients with diseased hearts.

In the present study we not only documented an effective and feasible technique for the rapid induction of mild (34°C) to moderate hypothermia (27°C) during CPCR steps A–B–C but also demonstrated that hypothermia significantly improves outcome without cardiovascular side effects during prolonged VF in a model of refractory CA. The finding that mild hypothermia is as effective as moderate hypothermia in preserving the viability of the organism obviates the need for lowering the temperature beyond the relatively safe limit of 34°C. We recognize that studies are needed to evaluate the effect of mild hypothermia on the ability to achieve ROSC without CPB in experimental models with diseased hearts. Recent clinical studies suggest that in patients with diseased hearts, postarrest mild hypothermia induced by infusion of large volume (30 mL/kg) of intravenous iced (4°C) crystalloids does not cause arrhythmias and may actually improve hemodynamics (29). Ideally, hypothermia should be induced immediately after CA. However, experience from clinical studies of out-of-hospital CA indicates that 8–10 mins is required in many urban areas for the ambulance to arrive and for paramedics to initiate ROSC attempts (2, 3, 30, 31). Therefore, in the present study the dogs were subjected to 3 mins of no flow, simulating the reaction time for a bystander, followed by 7 mins of BLS, to approximate the time required for the ambulance to arrive. Hypothermia was not induced until after another 10 mins at normothermia, simulating ROSC at-

Mild or moderate hypothermia during prolonged cardiopulmonary cerebral resuscitation in dogs preserves viability of extracerebral organs and improves outcome.

tempts and the time needed to gain vessel access.

Alternative explanations for the results of this study should be considered. The excellent neurologic outcomes in the hypothermia groups may, theoretically, have been the result of better perfusion pressures during CPR. Indeed, the flush administered at the start of the venovenous shunt cooling did increase blood pressure, similar to what has been seen clinically (29). The normothermic shunt group 4, however, had similar blood pressure responses as the hypothermia groups but had significantly worse outcome. Thus, it appears that hypothermia improved outcome. Similarly, the hypothermia groups had less myocardial damage than the normothermia groups. Hypothermia presumably had a protective effect on the heart. Mechanisms of this protection may include decreased heart rate, decreased oxygen demands, decreased apoptosis (32), and increased production of heat shock proteins (33). The resultant improved hemodynamics could have contributed to the improved neurologic outcome.

One potential criticism of the study is that the control (normothermic) group was actually actively warmed during CPCR. This was done to be sure that temperatures were tightly controlled during the insult. Clinically, however, patients tend to cool spontaneously during CA and CPCR. Thus, the protocol may have exaggerated the effects of mild hypothermia since the control group may have actually been hyperthermic compared with patients. The results of this study suggest that perhaps exposure of the victim or other measures should be used during CA to enhance this spontaneous cooling.

Limitations of this study also include the fact that the researcher who evaluated the functional outcome (OPC, NDS) could not be blinded for the treatment groups, although the pathologist who evaluated cerebral histologic outcome was blinded. These experiments require a large team of personnel, who consequently become aware of the group assignments. There were no other personnel available to routinely perform neurologic assessments in every animal. In previous studies, agreement between team members on outcomes scores was good. This is not surprising since the differences between the overall performance categories, the primary functional outcome variable, are not subtle. In addition, the fact that the histopathologic findings correlate with the OPCs suggests a lack of bias in OPC determination.

For clinical use, the catheters used in this study for the venovenous shunt cooling (34) could be replaced by a double-lumen central venous catheter, inserted through a large vein (basilic, cephalic, internal jugular, femoral), with inflow and outflow sites separated. In our pilot experiments we found that the venovenous cooling during CPR is less effective if the tips of the catheters are <20 cm apart. Another alternative is a standard central venous catheter for inflow into the heat exchanger and any peripheral venous catheter for delivery of cooled blood to the patient. Our improvised cooler (heparinized tubing in ice water) (34) might be replaced by a still-to-be-developed, FDA-approved miniaturized pump-cooler for field use. Although the 10 mins allowed in this protocol for achieving venous access and initiation of cooling seems unrealistic with standard techniques, we are working with catheter and imaging companies to develop novel approaches to vessel cannulation that could be more rapid and reliable, even in the hands of physician extenders.

CONCLUSIONS

We conclude that in normovolemic "refractory" VF-CA of 40 mins in dogs, cooling to mild (Tty 34°C) or moderate (Tty 27°C) hypothermia during ROSC attempts of 20 mins with external CPCR steps A–B–C (for BLS and ALS) as a bridge to prolonged CPB can result in survival with full neurologic recovery. This was not achievable in the same scenario with normothermic closed-chest CPCR, despite mild hypothermia after ROSC. A clinically acceptable portable device for blood cooling during CPCR should be developed.

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Suspended Animation Can Allow Survival without Brain Damage after Traumatic Exsanguination Cardiac Arrest of 60 Minutes in Dogs

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Background: **We have previously shown in dogs that exsanguination cardiac arrest of up to 120 minutes without trauma under profound hypothermia induced by aortic flush (suspended animation) can be survived without neurologic deficit. In the present study, the effects of major trauma (laparotomy, thoracotomy) are explored. This study is designed to better mimic the clinical scenario of an exsanguinating trauma victim, for whom suspended animation may buy time for resuscitative surgery and delayed resuscitation.**

Methods: **Fourteen dogs were exsanguinated over 5 minutes to cardiac arrest. Flush of saline at 2°C into the femoral artery was initiated at 2 minutes of cardiac arrest and continued until a tympanic temperature of 10°C was achieved. The dogs were then randomized into a control group without trauma (n = 6) or a trauma group (n = 8) that underwent a laparotomy and isolation of the spleen before hemorrhage and then, at the start of cardiac arrest, spleen transection and left thoracotomy. During cardiac arrest, splenectomy was performed. After 60 minutes of no-flow cardiac arrest, reperfusion with cardiopulmonary bypass was followed by intensive care to 72 hours.**

Results: **All 14 dogs survived to 72 hours with histologically normal brains. All control dogs were functionally neurologically intact. Four of eight trauma dogs were also functionally normal. Four had neurologic deficits, although three required prolonged mechanical ventilation because of airway edema and evidence of multiple organ failure. Blood loss from the chest and abdomen was variable and was associated with poor functional outcomes.**

Conclusion: **Rapid induction of profound hypothermic suspended animation (tympanic temperature, 10°C) can enable survival without brain damage after exsanguination cardiac arrest of 60 minutes even in the presence of trauma, although prolonged intensive care may be required. This technique may allow survival of exsanguinated trauma victims, who now have almost no chance of survival.**

Key Words: **Suspended animation, Brain damage, Traumatic exsanguination.**

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Despite advances in resuscitation techniques and in surgical management of trauma victims, survival rates remain extremely low in trauma patients who exsanguinate to cardiac arrest.[1,2] Emergency department thoracotomy to treat cardiac tamponade, control intrathoracic hemorrhage, perform open-chest cardiac massage, and cross-clamp the aorta to optimize cerebral and myocardial perfusion and decrease intra-abdominal hemorrhage is often performed, but the surgical team's race against the clock to achieve hemostasis is rarely successful, even when the underlying injury is technically repairable. Most patients die or suffer severe brain injury because these extraordinary efforts are not adequate to restore blood flow before the limit of tolerance under normothermia of 5 minutes of circulatory arrest for the brain[3,4] and approximately 20 minutes for the heart.[4,5]

In 1984, Bellamy et al. considered these issues when reviewing data from the Vietnam War. It was clear that a new approach to resuscitation is needed.[2] Suspended animation (i.e., rapid induction of pharmacologic-hypothermic preservation) was introduced as a new concept for attempting resuscitation from cardiac arrest in presently unresuscitable victims.[2,6] The viability of brain and organism is preserved with suspended animation during cardiac arrest to buy time for transport and resuscitative surgery, until restoration of spontaneous circulation or prolonged artificial circulation is possible. Using dog outcome models of exsanguination cardiac arrest, the Pittsburgh group has systematically explored suspended animation potentials. The initial studies included pressure-controlled hemorrhagic shock, rapid cooling by means of cardiopulmonary bypass (CPB), 60 to 120 minutes of deep (15°C) or profound (<10°C) circulatory arrest, and resuscitation by means of CPB.[7,8] Because CPB cannot be initiated rapidly enough, we have more recently explored

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induction of suspended animation by means of a rapid flush of ice-cold saline into the aorta. Hypothermic preservation induced within 5 minutes of circulatory arrest by means of aortic or femoral cold saline flush has allowed long-term survival without brain damage after up to 120 minutes of no-flow cardiac arrest.[9–12]

The experimental model in these previous studies involved exsanguination but not major tissue injury. However, the majority of patients who experience hemorrhage severe enough to cause exsanguination cardiac arrest have a major vascular or solid organ injury with significant tissue trauma. The potential efficacy of suspended animation in traumatic exsanguination cardiac arrest has therefore been questioned, as trauma may affect the distribution of preservative cold flush, cause coagulopathy, and elicit inflammatory and other deleterious responses. In the present study, we aimed to determine the outcome after suspended animation in a clinically realistic dog model of exsanguination cardiac arrest with abdominal injury and thoracotomy. Major tissue trauma like this has not been explored in previous studies by our group. We hypothesized that the addition of trauma would worsen the chance of intact survival.

MATERIALS AND METHODS

This study was approved by the Institutional Animal Care and Use Committee of the University of Pittsburgh and the Department of Defense and was conducted in accordance with the Animal Welfare Act and other federal statutes and national guidelines for the treatment of animals. All surgical procedures were performed by the same team in a designated veterinary surgical suite with sterilized instruments and aseptic procedures.

A total of 35 custom-bred hunting dogs (21–28 kg body weight; age, 8–12 months) were used; 16 were used for pilot experiments to determine the trauma, hemorrhage, and flush; 5 were used as blood donors; and 14 were used for the definitive study with exsanguination cardiac arrest of 60 minutes with or without laparotomy, splenic injury, and thoracotomy. Whole blood of donor dogs was collected into 1,000-mL bags with 50 mL sodium citrate through a 19-Fr right external jugular vein catheter inserted under general anesthesia and stored at 6°C for up to 7 days.

Preparation

The dogs were fasted overnight with free access to water. After sedation with ketamine (10 mg/kg administered intramuscularly), anesthesia was induced with halothane (2–4%) in N_2O/O_2 (50%/50%) through a cone mask. The dogs were then intubated and mechanically ventilated (Harvard Piston Ventilator model 613, Harvard Apparatus, South Natick, MA) with a tidal volume of 15 mL/kg and the rate adjusted to maintain a $Paco_2$ of 35 to 40 mm Hg. A positive end-expiratory pressure of 5 cm H_2O was applied. Anesthesia was maintained during preparation with halothane 0.5% to 1.5% in N_2O/O_2 (50%/50%) without neuromuscular blockade.

Temperature probes were inserted for measuring tympanic membrane, esophageal, and rectal temperatures. Tympanic temperature (Tty) was controlled at 37.5 ± 0.1°C with heating blankets and heating lamps before the insult. Gastric and bladder catheters were inserted. Dextrose 5% in 0.45% sodium chloride was administered at 5 mL/kg/h through a peripheral intravenous cannula (18 gauge) to ensure that the dogs were well hydrated and not hypoglycemic before the insult. A 10-Fr catheter was inserted into the left femoral artery for monitoring arterial blood pressure and blood sampling. A pulmonary artery catheter (7.5 Fr, Intellicath Continuous Cardiac Output Thermodilution Catheter, Baxter Co., Irvine, CA) was inserted through the left femoral vein and advanced into wedge position for pressure and temperature monitoring, cardiac output determination, and blood sampling. Arterial and central venous pressures and electrocardiogram were continuously recorded on a polygraph (Grass Model 7D Polygraph, Quincy, MA). Pulmonary artery pressure, pulmonary artery occlusion pressure, cardiac output, arterial and mixed venous blood gases, hemoglobin, hematocrit, serum sodium, potassium, glucose, and lactate levels were measured at regular intervals. Coagulation abnormalities were assessed using thromboelastography (Thromboelastograph, Hemoscope Co., Morton Grove, IL). Reaction time, clot formation time, alpha angle, and maximum amplitude were measured and recorded for each animal at baseline and at 1, 3, 6, 9, 15, 24, and 72 hours after reperfusion, using celite-activated whole blood at 37°C.

A 7- to 8-gauge cannula was inserted 3 cm into the right femoral artery for arterial cold flush after induction of circulatory arrest and, after cardiac arrest of 60 minutes, to return arterialized blood to the animal from the CPB circuit (CPB arterial cannula).[13,14] The right external jugular vein was cannulated with a multiple-hole 19-Fr catheter, which was advanced to the level of the right atrium, for venous bleeding during exsanguination and later for venous return to the CPB system.

Insult

In the trauma group, a midline laparotomy was performed providing exposure for the splenic injury and inducing tissue trauma just before hemorrhage (because trauma victims would have trauma before hemorrhage). The abdomen was temporarily closed with towel clips.

The model is illustrated in Figure 1. In both groups, after two baseline measurements, halothane and intravenous fluids were discontinued, heating devices were turned off, and the dogs were weaned to spontaneous breathing of N_2O/O_2 (70%/30%) through a T tube. When the canthal reflex returned, hemorrhage was initiated. Over a 5-minute period, the dogs were bled through the jugular venous cannula, and the blood was collected in 1,000-mL bags with 50 mL of sodium citrate for later reinfusion. The hemorrhage was controlled to achieve mean arterial pressure (MAP) of 40 mm Hg at 2 minutes, 30 mm Hg at 3 minutes, and 20 mm Hg at 4 minutes,

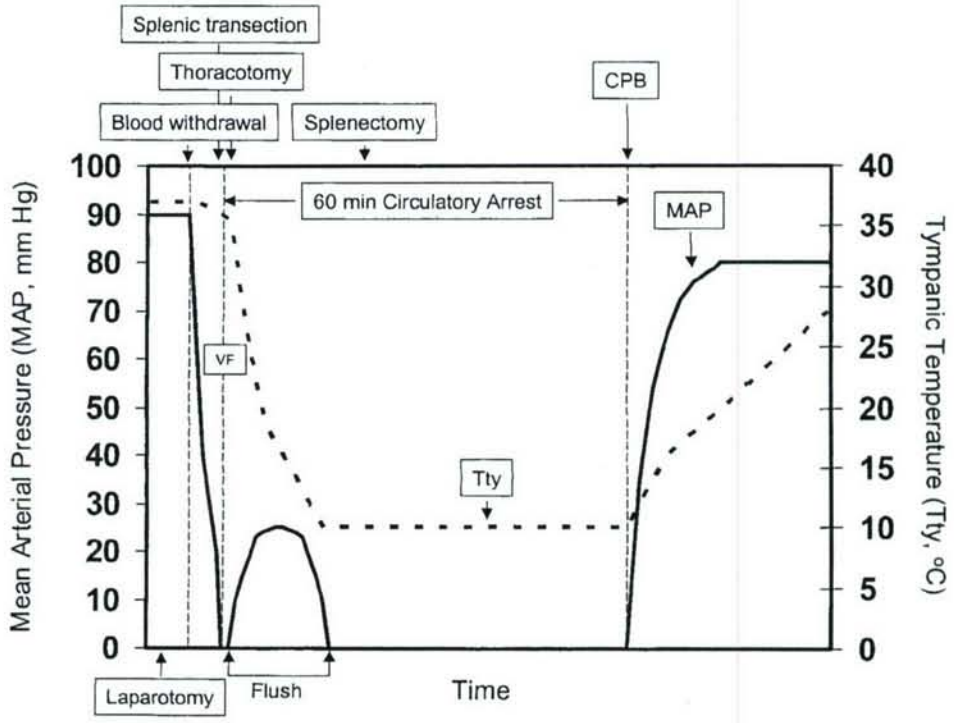

Fig. 1. *Experimental model for trauma and exsanguination, with preservation by cold aortic flush, 60 minutes of circulatory arrest, and resuscitation by means of cardiopulmonary bypass.*

at which time, in the trauma group, the abdomen was reopened and a standardized complete transection of the spleen was performed at its midpoint. At 5 minutes, to ensure zero blood flow, ventricular fibrillation was induced with a 95-V, AC, 60-Hz transthoracic shock through subcutaneous needles for 2 seconds and repeated as needed. Cardiac arrest was defined by identification of ventricular fibrillation on the electrocardiogram and the loss of arterial pulsation. Total arrest time (no-flow) was 60 minutes.

Preservation and Surgical Hemostasis

After 2 minutes of no-flow (cardiac arrest), normal saline at 2°C was flushed in both groups through the CPB arterial cannula into the femoral artery at a rate of 1.7 L/min, using a roller pump, until Tty reached 10°C. In the trauma group, at 2 minutes of no-flow, a left lateral thoracotomy (reproducing an emergency department thoracotomy) was performed at the sixth intercostal space, exposing the intrathoracic organs. At 20 minutes of cardiac arrest, simulating the time needed for transport to an operating room, splenectomy was performed. The abdominal wall and the thoracotomy incision were, however, left open to detect possible bleeding after initiation of CPB.

Resuscitation

Reperfusion after cardiac arrest no-flow of 60 minutes was achieved in both groups with CPB,[13,14] using heparin-coated circuits to avoid systemic heparinization (Carmeda-bonded circuits, Medtronic, Grand Rapids, MI). The CPB system was primed with 400 mL of lactated Ringer's solution with 2 mEq/kg of sodium bicarbonate. The flow was adjusted with a centrifugal pump (Biomedicus, Eden Prairie, MN) at 100 mL/kg/min. Reinfusion of the shed blood was titrated, aiming to maintain an MAP of 90 to 150 mm Hg and a central venous pressure of 10 to 15 mm Hg. If necessary, epinephrine boluses of 5 μg/kg were administered and norepinephrine infusion was titrated to maintain the MAP within the targeted range. Gas flow through the CPB oxygenator was adjusted to keep $Paco_2$ at 30 to 35 mm Hg. The temperature of the water bath of the CPB heat exchanger was set to 5°C above Tty, until Tty reached 34°C. Controlled ventilation was resumed with 100% oxygen, at a rate of 8 to 10 inspirations/min. The intravenous maintenance fluid was restarted with a flow of 100 mL/h. A base deficit of greater than 6.0 mEq/L was treated with sodium bicarbonate.

When pulmonary artery temperature reached 32°C, defibrillation attempts were initiated with external DC countershocks of 150 J, increased by 50 J for repeated shocks. If spontaneous circulation was restored, the CPB flow rate was reduced to 75 mL/kg/min at 60 minutes, to 50 mL/kg/min at 90 minutes, and was stopped at 120 minutes. Bleeding into the abdomen or chest was controlled with ligation of involved vessels and with electrocautery. The abdominal and thoracic incisions were closed in layers, with a left-sided chest tube (28 Fr) inserted through the seventh intercostal space along the midaxillary line. Donor blood was transfused, if neces-

sary, to maintain hematocrit above 25%. Partial crossmatch was accomplished before all transfusions by adding a drop of the donor blood to the recipient dog's blood at room temperature and observing for macroscopic agglutination.

Intensive Care

After weaning from CPB at 2 hours, controlled ventilation and circulatory support was continued to at least 20 hours. Neuromuscular blockade was maintained with intermittent doses of pancuronium bromide (0.1 mg/kg intravenously). Sedation and analgesia were provided with N_2O/O_2 (50%/50%) plus intravenous boluses of morphine (0.1–0.3 mg/kg) and diazepam (0.1–0.2 mg/kg) to prevent signs of wakefulness (e.g., mydriasis). Severe hypertension (MAP > 150 mm Hg) despite adequate analgesia was controlled with intravenous boluses of labetalol (0.25–0.5 mg/kg) or hydralazine (0.1–0.2 mg/kg).

Hypotension (MAP < 90 mm Hg) was treated with normalization of filling pressures by fluid administration (blood or 5% albumin depending on the hematocrit value) and with titrated norepinephrine. Standard intensive care included airway suctioning, periodic deep lung inflations, and position change (rotation). The dogs received cefazolin (250 mg intravenously) every 8 hours for infection prophylaxis.

At 20 to 24 hours, paralysis was reversed with neostigmine (50 μg/kg) plus atropine (25 μg/kg), and the dogs were weaned to spontaneous breathing through a T tube. The chest tube was removed in the trauma dogs after greater than 30 minutes of spontaneous breathing if signs of air leaks or ongoing blood loss were absent and if Pao_2 was maintained at greater than 100 mm Hg on air and $Paco_2$ was 30 to 40 mm Hg. The dogs were extubated when they met the above-mentioned criteria and after their upper airway reflexes had returned. If the dogs could not be weaned to spontaneous breathing or required continued circulatory support, they were kept ventilated for an additional 24 hours before new attempts at weaning. After extubation, the catheters were removed under brief N_2O-halothane anesthesia by cone mask. The dogs were transferred to a step-down unit to 72 hours, with oxygen by mask and continuous monitoring of pulse rate and arterial oxygen saturation. Suspected pain was controlled with titrated intravenous doses of morphine (0.1–0.2 mg/kg), and distress was controlled with intravenous diazepam (0.1–0.3 mg/kg). The maintenance fluid was dextrose 5% in NaCl 0.45% until 24 hours and dextrose 10% in NaCl 0.45% thereafter, until the dog was able to eat and drink. The dogs were continually monitored by technicians, with critical care physicians immediately available.

Outcome Evaluation

Performance was evaluated according to overall performance category (OPC), where 1 = normal, 2 = moderate disability, 3 = severe disability, 4 = coma, and 5 = death.[14] Neurologic function was evaluated as neurologic deficit scores (NDS), where 0% to 10% = normal and 100% = brain death.[3,14] OPC and NDS were evaluated every 8 hours after extubation. Attempts were made to discontinue any sedation at least 4 hours before final evaluations. If necessary, sedation was reversed with naloxone hydrochloride (narcotic antagonist) 1.5 to 6.0 μg/kg or with flumazenil (benzodiazepine antagonist) 0.1 mg intravenously, repeated if needed.

After final outcome evaluation, for morphologic studies, the dogs were reanesthetized with ketamine 10 mg/kg intramuscularly, followed by halothane 0.5% to 1.5% in N_2O/O_2 (50%/50%). A left thoracotomy was performed and the proximal descending aorta was ligated. A large-bore cannula was inserted proximal to the ligature. The dogs were then killed by infusing paraformaldehyde (4%, pH 7.4) into the aortic arch using a roller pump at a pressure of approximately 100 mm Hg, with the right atrium opened, until clear fluid returned. A complete necropsy was performed with scoring of macroscopic damage to extracerebral organs (minimal, mild, moderate, or severe), taking into account the pattern, appearance, and anatomic distribution of the lesions. One hour after perfusion fixation, the brain was removed. After cutting 3-mm-thick slices, the same six slices of each brain were paraffin-embedded, cut into sections 4 μm thick, and stained with hematoxylin-eosin-phloxine. Using light microscopy, the same pathologist, blinded for treatment assignments, scored 19 distinct anatomic brain regions for severity and extent of ischemic neuronal changes, infarcts, and edema, as described previously.[3] The total brain histologic damage score (HDS) was the sum of all area scores. An HDS of > 40 represents moderate damage and an HDS of > 100 represents severe damage.

Statistical Analysis

Data are presented as mean and SD unless otherwise stated. Repeated-measures analyses of variance were performed followed by Bonferroni/Dunn post hoc tests to identify differences in hemodynamic parameters and temperature data between groups over time. NDS and HDS scores were analyzed using Mann-Whitney *U* Test, and Fisher's exact test was used to assess differences in OPC proportions (OPC of 1 and 2, good outcome; vs. OPC of 3, 4, or 5, bad outcome) between groups. Pearson correlation coefficient was computed between the OPC and the volume of transfused blood, followed by Fisher's r to z transformation of the correlation coefficient to calculate a probability level. A value of $p < 0.05$ was considered statistically significant.

RESULTS

Pilot Experiments

Suspended animation induced by direct aortic (cannulation of the descending thoracic aorta through a left thoracotomy) flush of cold saline in a model of traumatic (laparotomy, liver, or spleen trauma) exsanguination cardiac arrest no-flow of 90 minutes consistently resulted in severe coagulopathy with flat TEG curves and rapid exsanguination from the vascular or soft tissue injuries, or multiple organ dysfunc-

Table 1 **Resuscitation Variables Required for Restoration of Spontaneous Circulation***

Group	Trauma		Control	
Countershocks, total number	1	(1–4)	1	(1–3)
Countershocks, total energy (J)	225	(150–700)	150	(150–450)
Time of ROSC†	43	(27–72)	47	(24–55)
Total bicarbonate (mEq)	97	(56–210)	111	(50–258)
Total epinephrine (mg)	0.9	(0.6–1.9)	1.5	(0.4–3.0)
Total norepinephrine (mg)	1.9	(0–5.9)	2.7	(1.2–7.8)

ROSC, restoration of spontaneous circulation.

* Data are represented as median (range).

† Time after initiation of cardiopulmonary bypass. No differences were observed between the groups.

tion (cardiovascular failure, respiratory failure, renal failure, and neurologic failure[15,16]). In other pilot experiments with abdominal and thoracic trauma, the flush was initially cephalad through a balloon catheter (8 Fr, Cardeon Co.) placed in the midthoracic aorta through the femoral artery until the target Tty reached 10°C, and then in a caudad direction by deflating the balloon and compressing the proximal aorta manually (by means of thoracotomy). After 90 minutes of cardiac arrest with trauma, all dogs died within 24 hours of irreversible shock. In contrast, in experiments without trauma and the same exsanguination insult, 90 minutes of no-flow cardiac arrest, and resuscitation, aortic flush through a catheter in the iliac artery resulted in good outcome.[12] With resuscitation from traumatic exsanguination cardiac arrest of 90 minutes not yet feasible in our model, an arrest duration of 60 minutes was chosen for the definitive study.

Resuscitation

All 14 dogs in the final series (both groups) were successfully resuscitated and survived to 72 hours. Restoration of spontaneous circulation was achieved within 70 minutes of recirculation with CPB (Table 1). There were no differences between the groups in requirements of drug dosages during CPB, in the number of countershocks, or in the energy delivered to achieve restoration of spontaneous circulation. Three dogs in the trauma group could not be weaned from controlled ventilation because of severe airway edema (resulting in upper airway obstruction) and spontaneous hypoventilation. Consequently, neurologic outcome was evaluated in these dogs at 72 hours after reversing the neuromuscular blockade and analgesia with the orotracheal tube in place and, if necessary, with intermittent hand ventilation. They were then reanesthetized for perfusion fixation and morphologic evaluation.

Physiologic Parameters

No significant differences were found in the baseline physiologic parameters between the two groups. Heart rate, MAP, and cardiac output values were not different between the groups (Table 2). There were no group differences in arterial pH, Po_2, Pco_2, or base excess during the experiment (controlled parameters). An average flush volume of 620 mL/kg (range, 360–800 mL/kg) was required to reach the target Tty of 10°C (Fig. 2). Lactate levels peaked in both groups 60 to 90 minutes after initiation of CPB without any group differences and returned gradually to baseline at approximately resuscitation time 6 hours.

Coagulation and Blood Loss

After initial exsanguination cardiac arrest, there was minimal blood loss from skin incisions. To maintain hemat-

Table 2 **Physiologic Variables in Trauma and Control Groups during Resuscitation from 60-Min Exsanguination Cardiac Arrest and Suspended Animation by Aortic Flush***

Time of Reperfusion	Mean Arterial Pressure (mm Hg)		Heart Rate (beats/min)		Cardiac Output (L/min)	
	Trauma	Control	Trauma	Control	Trauma	Control
Baseline	110 ± 16	116 ± 16	118 ± 13	121 ± 13	3.1 ± 1	2.6
5 min	77 ± 20	65 ± 19				
15 min	73 ± 6	77 ± 20				
30 min	83 ± 26	93 ± 16				
60 min	106 ± 7	118 ± 11	119 ± 29	132 ± 13		
90 min	123 ± 14	120 ± 18	124 ± 28	136 ± 30		
2 h	115 ± 19	118 ± 16	143 ± 16	139 ± 5	3 ± 1.1	3.7 ± 1.1
3 h	135 ± 21	122 ± 18	134 ± 22	138 ± 28	4.4 ± 1.1	4.3 ± 2.1
4 h	133 ± 13	135 ± 16	134 ± 16	128 ± 23	3.8 ± 1.2	3.9 ± 1.3
6 h	136 ± 10	143 ± 13	128 ± 16	118 ± 7	2.6 ± 1	2.6 ± 0.4
9 h	128 ± 16	127 ± 16	124 ± 26	115 ± 25	1.7 ± 0.9	2.4 ± 0.4
12 h	118 ± 15	126 ± 5	125 ± 42	102 ± 32	2.2 ± 0.9	2.7 ± 0.6
16 h	107 ± 11	123 ± 12	138 ± 43	113 ± 37	3.1 ± 0.4	2.7 ± 0.3
20 h	104 ± 15	106 ± 13	139 ± 47	104 ± 37	2.9 ± 0.5	2.9 ± 0.7
24 h	98 ± 11	93 ± 11	138 ± 41	90 ± 28	3 ± 0.7	

BL, baseline.

* Values are expressed as mean ± SD. No heart rate values available during cardiac arrest. No cardiac output values available during cardiopulmonary bypass. No differences were observed between groups.

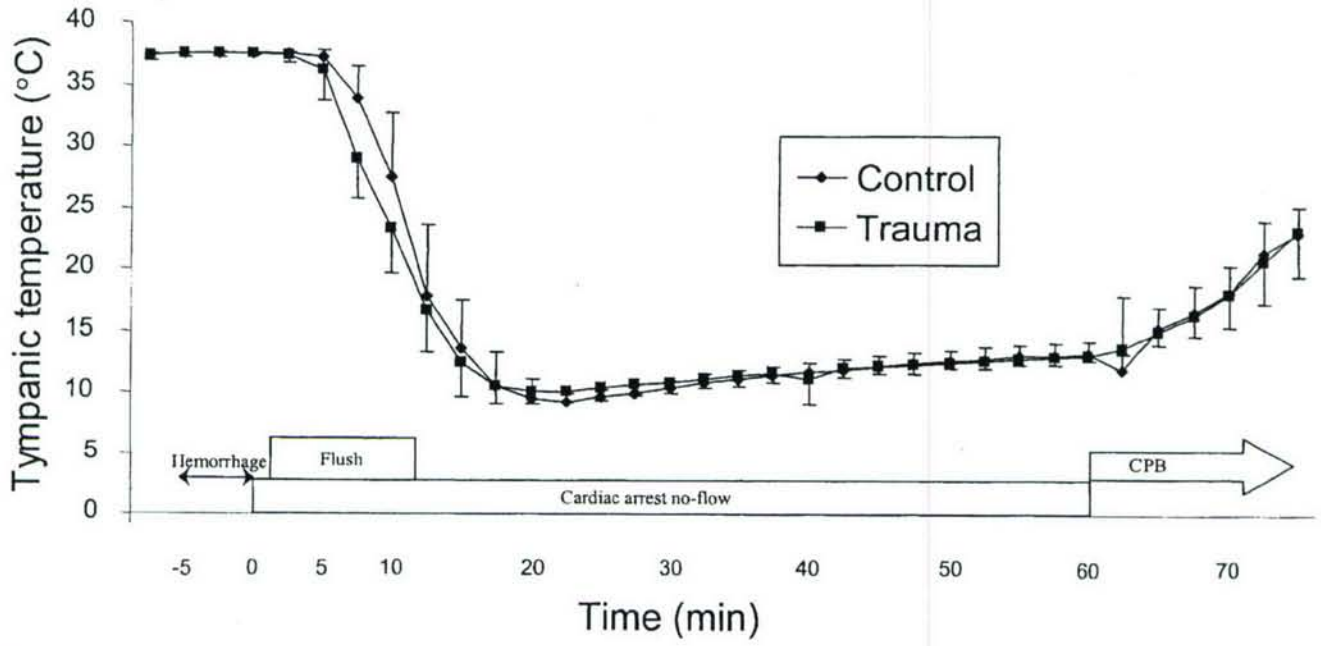

Fig. 2. *Tympanic membrane temperatures during exsanguination cardiac arrest of 60 minutes of no-flow. Resuscitation was with cardiopulmonary bypass. Data are given as mean ± SD. No differences were observed between the groups.*

ocrit above 25%, transfusion of donor blood was, however, required in six of eight dogs in the trauma group and in no dogs in the control group. Blood loss from the abdominal or thoracic injuries varied (Table 3). The transfusion volume varied between 0 and 1,500 mL and correlated with final neurologic deficit ($p = 0.039$) (i.e., the greater the need for blood, the worse the neurologic deficit). Both groups demonstrated coagulation abnormalities after the insult, with transient hypocoagulability by TEG (decreased alpha-angle and decreased maximum amplitude) at 1 hour of recirculation (Table 4); TEG variables normalized in both groups within 24 hours but indicated a hypercoagulable state at the end of the experiment (72 hours), with an increased alpha-angle and large maximum amplitude.

Extracerebral Outcome

At 72 hours, in both groups, arterial pressure (Table 2) and blood gas values were normal and no dog required norepinephrine. At necropsy, moderate edema of subcutaneous tissue, airway mucosa, and intestinal mucosa was observed in two trauma dogs and mild to moderate pleural effusions and ascites in four of the eight trauma dogs (Table 5).

Table 3 Cumulative Blood Loss and Transfusion Volume (mL) in the Trauma Dogs*

	Dog 1		Dog 2		Dog 3		Dog 4		Dog 6		Dog 8	
	Blood Loss	Transfusion	Blood Loss	Transfusion	Blood Loss	Transfusion	Blood Loss	Transfusion	Blood Loss	Transfusion	Blood Loss	Transfusion
Time of reperfusion (h)												
1											500	400
2									125		1,150	1,200
4									255	400	1,250	
6	60		550		0		70			800		
9					350				570			
12			950	750			160		935			
18	115				750	550				1,400		
24	176		1,300		800	950			1,275			
40	250					1,400			1,325	1,500		
Major bleeding site			Chest		Abdomen				Chest		Abdomen	
OPC		1		3		4		2		4		1

OPC, overall performance category.

* There was no significant bleeding in dogs 5 and 7, both of which had OPC 1 at 72 h.

Table 4 Thromboelastography Variables in Trauma and Control Groups

Time (h)	Alpha-Angle		Reaction Time (min)		Coagulation Time (min)		Maximum Amplitude (mm)	
	Trauma	Control	Trauma	Control	Trauma	Control	Trauma	Control
Baseline	60 ± 13	60 ± 9	6.3 ± 2.0	6.3 ± 1.2	10.7 ± 7.8	8.9 ± 2.1	54 ± 12	60 ± 9
1	32 ± 13*	42 ± 19*	9.4 ± 1.8*	9.3 ± 3.7	20.0 ± 11.1*	16.1 ± 7.7*	37 ± 11*	45 ± 11*
3	49 ± 16*	45 ± 16*	8.1 ± 2.2*	9.9 ± 6.0	18.5 ± 20.4*	16.5 ± 12.1	50 ± 14	49 ± 12
6	42 ± 15*	51 ± 9*	9.8 ± 4.1	8.1 ± 3.1	21.8 ± 18.6*	12.4 ± 3.8*	48 ± 10	49 ± 13
9	42 ± 18*	49 ± 10*	9.3 ± 4.2*	8.4 ± 4.4	13.1 ± 3.9	12.7 ± 6.4	42 ± 17*	53 ± 7
15	45 ± 9*	53 ± 11*	8.0 ± 2.3	7.3 ± 1.3	13.0 ± 3.3	11.4 ± 2.5	50 ± 6	49 ± 15
24	55 ± 6	51 ± 12	6.7 ± 1	8.6 ± 2.4	10.2 ± 1.8	12.8 ± 3.9	54 ± 3	48 ± 11
72	67 ± 13	71 ± 3*	5.9 ± 3.2	7.0 ± 1.4	8.0 ± 4.3	8.8 ± 1.8	68 ± 7	70 ± 3

* $p < 0.05$ vs. baseline. No significant differences were observed between the groups. Values are expressed as mean ± SD.

In the control group, no tissue edema and no other macroscopic extracerebral organ damage was observed at necropsy except for mild myocardial lesions (mainly focal subendocardial infarctions) in three of the six dogs. In six of eight dogs in the trauma group, macroscopic cardiac damage was present, especially involving the anterolateral free wall of the right ventricle. In two of eight dogs in this group, these lesions consisted of mild to moderate hemorrhagic infarctions mainly restricted to the subendocardium and subepicardium. In four trauma dogs, the heart surface lesions had coalesced and focally extended to transmural involvement. In the trauma group, total serum creatinine kinase significantly increased to 727 IU/L (range, 447–1,593 IU/L), but there was no significant increase in the creatine kinase-MB isoenzyme proportion and no increase in troponin-I levels, except for one dog with troponin-I of 8.8 ng/mL.

The lungs in both groups appeared normal, except hemorrhagic consolidation in one lower lobe in one trauma dog. The intestinal mucosa had mild to moderate hemorrhagic areas in three dogs in the trauma group. Anuria started in both groups with the onset of cardiac arrest and ended after 30 to 60 minutes of reperfusion, except in one trauma dog in which it persisted until 20 hours; oliguria persisted in this dog as the creatinine level increased to 6.8 mg/dL and blood urea nitrogen increased to 66 mg/dL at 72 hours. The kidneys had focal infarctions, edema, or hemorrhage in four dogs in the trauma group. At 72 hours, serum aspartate aminotransferase values were significantly increased in both groups (median, 126 IU/L; range, 45–865 IU/L), whereas γ-glutamyl transpeptidase and bilirubin concentrations remained normal and serum albumin was below normal (median, 2.3; range, 2.1–2.5 g/dL).

Cerebral Outcome

Final OPCs at 72 hours were better in the control group (Fig. 3). All six control dogs were functionally normal (OPC 1). In the trauma group, five of eight dogs were neurologically intact or had minor deficits (OPC 1 or 2). Three dogs in

Table 5 Gross Extracerebral Organ Damage at Necropsy (72 h) after 60-Min Exsanguination Cardiac Arrest and Resuscitation*

Group	Dog	Edema	Pleural Effusion	Ascites	Heart	Lungs	GI Tract	Kidney	Liver
Trauma	1	0	0	1	1	0	2	0	0
	2	0	0	0	2	0	1	0	0
	3	4	1	0	4	0	0	1	0
	4	0	0	0	0	0	0	0	0
	5	0	2	1	0	1	0	0	0
	6	0	0	0	4	0	0	3	0
	7	3	2	0	4	0	3	2	0
	8	0	3	0	0	0	0	2	0
Control	1	0	0	0	0	0	0	0	0
	2	0	0	0	1	0	2	0	0
	3	0	0	0	1	0	0	0	0
	4	0	0	0	0	0	1	0	0
	5	0	0	0	0	0	0	0	0
	6	0	0	0	2	0	0	0	0
Trauma group (n = 8)		0 (0–4)	1 (0–3)	0 (0–1)	2 (0–4)	0 (0–1)	0 (0–3)	1 (0–3)	0
Control group (n = 6)		0	0	0	0 (0–2)	0	0 (0–2)	0	0

GI, gastrointestinal.

* 1 = minimal, 2 = minor, 3 = moderate, and 4 = severe. Group values are expressed as median (range).

this group had poor neurologic outcome ($p = 0.208$): two remained comatose (OPC 4) and required controlled ventilation despite discontinuation of anesthesia, sedatives, and analgesics and despite administration of naloxone and flumazenil; the third was reintubated within an hour after extubation at 24 hours because of stupor, general weakness, and respiratory failure. The latter dog also remained on controlled ventilation until 72 hours. Final NDS was normal in the control group (median, 1; range, 0–13) and abnormal in four of the eight trauma dogs (median, 12; range, 0–87) ($p = 0.004$) (Fig. 3). Histologically, total brain HDS at 72 hours was near normal in all dogs of both groups and averaged 12 (range, 4–22) in the control group versus 0 (range, 0–6) in the trauma group (not significant) (Fig. 4). Regional brain HDS had the same distribution in both groups, with putamen and caudate nucleus being the most vulnerable regions. Histopathologic changes consisted mainly of scattered ischemic neurons in the vulnerable areas and, in three dogs, mild edema with no infarction. There were no significant differences between groups.

DISCUSSION

In pilot experiments, we found that exsanguination cardiac arrest of 90 minutes plus trauma is not reversible to intact survival, whereas without trauma full neurologic recovery could be achieved.[12] The cause of early postarrest death in the trauma experiments with 90 minutes of cardiac arrest, despite standard life support, was failure of multiple extracerebral organs, without significant brain damage. In the present definitive study, using a dog model of traumatic exsanguination cardiac arrest of 60 minutes, we found that rapid induction of profound hypothermia (suspended animation) can enable survival without brain damage, as we have shown without trauma in a previous study.[12] Although postresuscitative extracerebral organ complications were worse in the trauma group, all the dogs survived to 72 hours, five of eight with good overall performance (OPC 1 or 2).

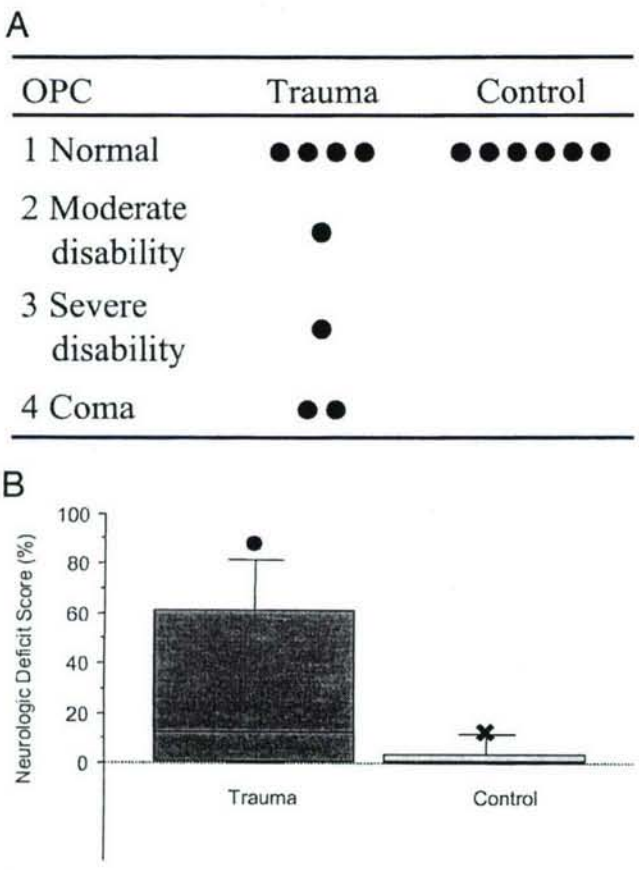

Fig. 3. *Final overall performance categories (OPC 1–5,* A*) and neurologic deficit scores (NDS,* B*) at 72 hours after exsanguination cardiac arrest with or without trauma. Each dot of OPC represents one dog. NDS is depicted in a box plot with the box containing the 25th to 75th percentiles and the horizontal line representing the median. The whiskers represent the 10th and 90th percentiles. Outliers are plotted separately as the* filled circle *(trauma group) or the* × *(control group).*

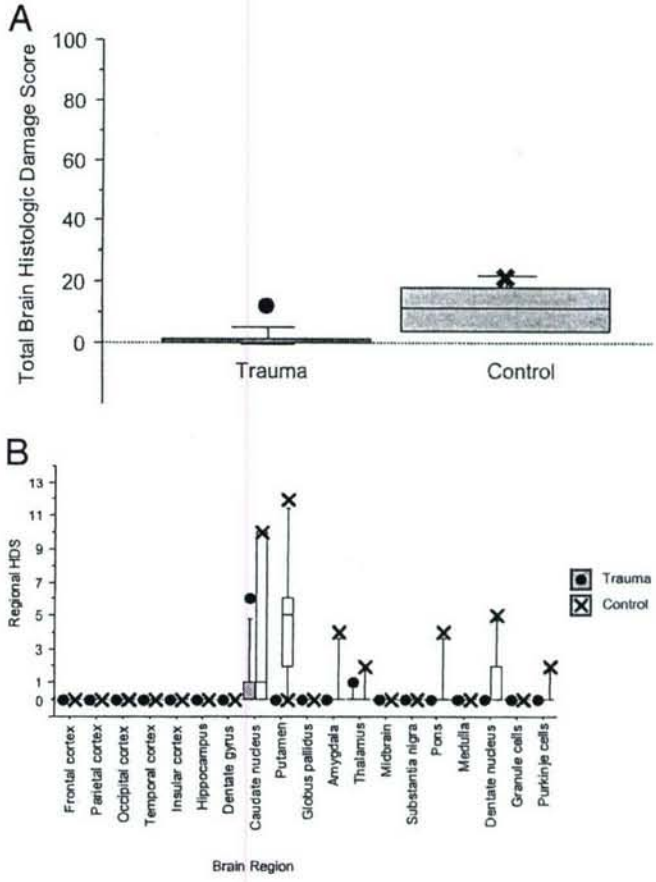

Fig. 4. *Total* (A) *and regional* (B) *brain HDS at 72 hours after exsanguination cardiac arrest with or without trauma. HDS is depicted in a box plot, with the box containing the 25th to 75th percentiles and the horizontal line representing the median. The whiskers represent the 10th and 90th percentiles. Outliers are plotted separately as the* filled circle *(trauma group) or the* × *(control group).*

Most importantly, no dog, with or without trauma, had any significant morphologic damage to the brain. This finding is important because, with conventional resuscitation techniques, the prognosis after traumatic exsanguination cardiac arrest is extremely poor.[1,17,18] The lack of histologic brain damage suggests that with longer intensive care life support (beyond 72 hours), as is available clinically, all dogs might have achieved normal overall function despite trauma. Such expensive long-term intensive care is not feasible in the laboratory.

The concept of preserving the organism with suspended animation to buy time for transport and surgical repair with delayed resuscitation particularly applies to civilian or military trauma victims with truncal injuries who exsanguinate to cardiac arrest without concomitant brain injury. Such casualties are considered unresuscitable despite the fact that their injuries are technically repairable.

Systematic outcome studies in dogs have documented the feasibility of suspended animation for delayed resuscitation from cardiac arrest no-flow periods of up to 120 minutes.[9–12] Cerebral temperature of 10°C was needed to preserve the brain beyond 60 minutes of no-flow. The model used in these earlier studies with exsanguination through arterial and venous catheters simulated isolated vascular injuries, without major tissue trauma. The majority of exsanguinating trauma victims, however, have concomitant injury to soft tissues and solid organs. Trauma may cause the systemic inflammatory response syndrome, including release of cytokines, soluble adhesion molecules, and oxygen free radicals leading to lipid and protein oxidation, which is associated with the development of the multiple organ dysfunction syndrome (MODS).[19–21] Moreover, coagulation disturbances associated with trauma, ischemia, hemodilution, hypothermia, cardiopulmonary bypass, and reoxygenation injury may impact the outcome of operative intervention and may decrease the chance of achieving surgical hemostasis and long-term survival.[22–25] In the present model, all these pathophysiologic disturbances are associated with suspended animation and may contribute to the development of severe coagulopathy and MODS. The extracerebral organ complications observed in the three dogs of the trauma group with poor outcome (OPC 3 and 4) are characteristic of MODS as defined by physiologic criteria.[15,16] Despite these dogs' poor overall and neurologic performance, however, no histologic damage was found in their brains. This discrepancy between histologic brain damage and clinical performance is in contrast with our results from previous cardiac arrest studies without trauma, in which a significant correlation has been seen between NDS and HDS.[4,7–11,13,25–31] Extracerebral organ dysfunction was, however, not present in the previous studies. Because the frequency of extracerebral organ dysfunction was not sufficiently anticipated in this study, laboratory values to quantify the levels of dysfunction were not performed, but should be in future studies. In addition, exploration of the various chemical cascades that may lead to MODS is warranted.

Poor OPC and NDS scores in the trauma dogs of the present study may represent a metabolic encephalopathy, which is potentially reversible if the underlying derangement is corrected. These dogs continued to require ventilatory support. To tolerate this, they needed sedation and intermittent doses of a neuromuscular blocker. We cannot be certain that these effects were totally reversed before the 72-hour evaluation of function.

The finding that the need for blood transfusion was associated with worse functional outcome suggests that common pathophysiologic mechanisms are involved in the initiation of coagulation derangements and MODS. In contrast, one could postulate that the blood transfusions had a direct effect on neurologic outcome, although the pathophysiologic mechanism for this would have to be explored. Additional studies focused on coagulation derangements are planned. Given the findings related to transfusions, the amount of donor blood transfused may need tighter control.

Limitations of this study include the small number of dogs used, which may not detect small differences in the analyzed parameters. Although our trauma model is clinically relevant, it does not represent the wide spectrum of tissue injury that may cause exsanguination cardiac arrest. In addition, intensive care was provided for a maximum of 72 hours, whereas the three trauma dogs with poor outcome would have required intensive care beyond this period of time in clinical practice. Given the lack of histopathologic brain damage, it is likely that prolonged intensive care would have led to good outcomes in these dogs. Unfortunately, continuing intensive care in these animals, for days or weeks, would be logistically and financially difficult.

The clinical relevance of a flush at 2 minutes of no-flow could be questioned. In a previous study,[32] we found that delaying the flush to 8 minutes worsened neurologic outcome. We believe that, in the emergency department, a trauma surgeon could open the chest and, with novel catheters, cannulate the aorta within 2 minutes of the loss of pulse. We are working with companies to develop such catheters. Even with a simple device like a Foley catheter, Rhee et al.[33] rapidly induced suspended animation through a thoracotomy and aortotomy. At the same time, we are exploring more optimal fluids and pharmacologic approaches[29–31] to lengthen the "window of opportunity" for induction of suspended animation. Other details, such as the rate of cooling, deserve further study, particularly because Alam et al. (personal communication) recently found that more rapid cooling is advantageous. These studies are part of our systematic research project to develop suspended animation.

Because suspended animation of 1 hour can be survived without evidence of histologic brain damage despite extracerebral trauma and MODS, clinical feasibility trials of suspended animation for victims of exsanguination cardiac arrest should be considered, starting in large trauma centers. Potential subjects would be trauma victims who have a mechanism of injury consistent with exsanguinating hemorrhage and lose a pulse just before, or after, arrival in the emergency department. These patients typically undergo a resuscitative thora-

cotomy. Rapid access to the descending aorta could be obtained directly and ice-cold saline could be flushed toward the heart and brain.[9–12]

In conclusion, suspended animation in dogs, using aortic cold flush and delayed resuscitation with cardiopulmonary bypass, enables survival without brain damage after exsanguination cardiac arrest of 60 minutes of no-flow, even in the presence of trauma. Extracerebral organ complications after resuscitation, however, are worsened by trauma. Consequently, long-term intensive care life support will be needed.

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THERAPEUTIC HYPOTHERMIA

Edited by

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Chapter 16

FUTURE DIRECTIONS

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Hypothermia research has come a long way over the past 50 years. We have asked many questions about how hypothermia works and in which situations is it beneficial. From a mechanistic standpoint, our understanding of the effects of hypothermia, both beneficial and detrimental, are much more complex than just direct effects on oxygen metabolism, as was first thought.

Laboratory studies have demonstrated benefit of therapeutic hypothermia in cardiac arrest caused by ventricular fibrillation, asphyxiation, or exsanguination, as well as traumatic brain injury, stroke, hemorrhagic shock, myocardial infarction, hepatic failure, and even pulmonary failure with sepsis. Additional studies are needed to more clearly define the optimal timing of hypothermia in terms of induction and duration, and the optimal depth, which are likely to be different for each insult. As we move forward in translating these findings to clinical studies, there may be appropriate indications to perform studies, if not done already, in animals that are high on the phylogenetic scale to hopefully better predict what will happen in humans.

The pathophysiologic mechanisms behind the molecular, cellular, organ, and organism level changes that occur after the various disease states addressed in this book are quite complex. So far, however, there has been little research into multifaceted therapeutic interventions that include hypothermia. Better understanding of the biochemical and molecular mechanisms behind the effects of hypothermia should allow us to develop synergistic pharmacologic approaches that perhaps could complement or potentate the beneficial affects of hypothermia, as well as decrease the

pathway will have an overall beneficial affect with such complex disease processes in critically ill patients. It is not surprising that so many single drug clinical trials have failed to show benefit.

Hypothermia affects many pathways simultaneously, and thus may have more hope for benefit. The fact that two randomized controlled clinical trials demonstrated benefit of hypothermia in diverse groups of patients after cardiac arrest, even when induced relatively slowly, is encouraging, particularly since drug studies have been disappointing. This success should help the endeavors to proceed with clinical trials after other insults. By the same token, the overall negative results of the most recent trial of hypothermia after head injury should not discourage further studies in this area. We should learn lessons from this study regarding the potential for different outcomes with different subpopulations, the need for close attention to detail regarding protocolized patient care algorithms, and timing of hypothermia induction.

Even when positive studies are published, clinical research should not stop there. Additional studies are needed to improve the techniques for inducing hypothermia and to better define the optimal parameters, timing and depth. Ideally, we should develop key endpoints for resuscitation that help us decide when to cool, how deep, and when to rewarm. Patient selection should also be better defined.

Dissemination of research findings and development of clinical practice guidelines related to hypothermia become the next steps. It was impressive how quickly the American Heart Association and the International Liaison Committee on Resuscitation picked up on the studies showing benefit of hypothermia after cardiac arrest and published statements encouraging the use of hypothermia.

We've come a long way in laboratory and clinical research into the potential benefits of therapeutic hypothermia in a variety of situations. With this momentum, we should anticipate that hypothermia will be applied to even more disease states, perhaps ones that have not even been thought of yet. Rigorous research from the molecular level to the bedside needs to continue. Although hypothermia may not have the scientific appeal of the most recently described cytokine pathway, monoclonal antibody, or genetically engineered animal, funding agencies need to recognize the great potential for hypothermia to have impact upon complex disease states in acute care medicine. There is no doubt that hypothermia, when applied in the appropriate manner at the appropriate time, can help save lives.

Chapter 5

TRAUMATIC BRAIN INJURY: LABORATORY STUDIES

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INTRODUCTION

This chapter will address the use of therapeutic hypothermia in traumatic brain injury (TBI). Hypothermia has a long-standing history of clinical use in the management of patients with severe TBI, specifically as a second tier therapy in the treatment of refractory intracranial hypertension. The resurgence in the interest in the use of mild and moderate hypothermia in experimental cerebral ischemia and cardiac arrest in the late 1980s and early 1990s (culminating in its recent successful translation to clinical use for cardiac arrest) prompted parallel investigation in TBI beginning in 1991. This chapter will focus on that work—specifically, both laboratory and clinical investigation in the use of therapeutic hypothermia in TBI since 1991.

The potential value of therapeutic hypothermia in the treatment of patients with severe TBI was suggested by Charles Phelps as early as 1897 (1), and may have even earlier roots. Phelps, in his classic treatise on "Traumatic Injuries of the Brain and its Membranes" recommended the application of an "ice cap" which, with the exception of trephination, he viewed as the most "directly curative resource." A number of reports in the mid 20th century suggested beneficial effects of hypothermia in patients with severe TBI (2-6) but these were not controlled clinical trials. Rosomoff reported on the use of moderate hypothermia in experimental TBI in a series of studies in dogs in the late 1950s and early 1960s (7-9). Beneficial effects of hypothermia on both mortality rate and specific secondary injury

mechanisms, such as local cerebral inflammation, were shown. State-of-the-art reviews by central nervous system (CNS) trauma experts written in the 1960s and early 1970s, such as Langfitt et al (10), clearly document that moderate hypothermia had become part of the routine clinical treatment of patients with severe TBI, particularly those with intracranial hypertension. Even in the classic first report of the use of continuous intracranial pressure (ICP) monitoring in patients with severe TBI, by Lundberg et al (11), moderate hypothermia was already an integral component of the standard treatment regimen. In the early 1980s, moderate hypothermia gradually fell out of favor--likely as a result of infectious complications associated with its prolonged use. Documentation of complications with the prolonged use of moderate hypothermia, although limited, was most convincing in the literature on management of pediatric victims of near-drowning accidents (12, 13). These reports, however, greatly influenced clinicians treating patients with severe TBI, along with other CNS insults, and therapeutic hypothermia was gradually abandoned (14).

STUDIES OF THERAPEUTIC HYPOTHERMIA IN LABORATORY MODELS OF TBI

Following the resurgence of interest in the application of mild hypothermia in experimental incomplete cerebral ischemia in rats and cardiac arrest in dogs, reports began to resurface on the beneficial effects of both moderate and mild hypothermia in experimental TBI. In 1991, Clifton et al (15) studied the effect of moderate (30°C) and mild (33°C) hypothermia after fluid percussion injury in rats. Rats were cooled before injury and maintained at target temperature for only 1 h. Despite this rather brief period of hypothermia, motor deficits -- assessed over several days posttrauma -- were attenuated versus those seen after TBI in normothermic rats. In Clifton's report, mild hypothermia was shown to be effective, but moderate hypothermia was even more beneficial. Mortality rate was reduced by moderate hypothermia vs. that seen in rats treated with either mild hypothermia or normothermia. This publication was followed by a flurry of over 40 reports from numerous laboratories from the early 1990s to the present investigating the effects of hypothermia on cellular and molecular mechanisms, intracranial dynamics, and outcome in experimental TBI. Over 90% of these reports have demonstrated a beneficial effect of hypothermia in experimental TBI (Table 5-1).

Table 5-1. Synopsis of selected studies of therapeutic hypothermia in experimental traumatic brain injury since 1991

Date (Citation)	*Species/ Model*	*Temperature*	*Outcome parameter(s)*	*Key Finding(s)*
1991 (15)	Rat/[1]FPI	30°C, 33°C	Motor function and mortality	Hypothermia reduced motor deficits and mortality, 30°C better than 33°C
1992 (29)	Rat/FPI	30°C	[2]BBB	Hypothermia reduced BBB injury
1993 (51)	Rat/FPI	30°C	[3]MAP2	Hypothermia reduced MAP2 loss
1993 (52)	Rat/FPI	30°C	Motor function	Hypothermia reduced motor deficits, 30 min treatment window
1993 (16)	Rat/FPI	30°C	[3]CSF [4]ACh levels	Hypothermia reduced the increase in ACh
1993 (47)	Rat/CCI	32-33°C	Histology [5]EAA levels by microdialysis	Hypothermia reduced lesion volume but not EAA levels
1993 (23)	Dog/Epidural balloon	31°C 5 h 35 °C 5-62 h	[6]ICP and histopathology	31°C reduced ICP
1994 (27)	Rat/FPI	30°C	Histopathology	Hypothermia reduced cell loss in CA3 and CA4 loss and contusion volume
1994 (53)	Rat/ Contusion		[7]SOD [8]mRNA	No effect
1995 (43)	Rat/[9]CCI	32°C	[10]IL-1 and [11]NGF mRNA	Increases in both outcome parameters were reduced by hypothermia
1995 (54)	Rat/FPI	30-31.5°C	[12]HSP72	Increase in HSP72 reduced by hypothermia
1995 (17)	Rat/FPI	30°C	Motor and cognitive function	Hypothermia reduced functional deficits
1995 (46)	Rat/FPI	30°C	Glutamate [13]DHBA	Hypothermia reduced the increases in both outcome parameters
1996 (30)	Rat/CCI with hypotension	35.5°C	BBB	Hypothermia reduced BBB damage
1996 (22)	Immature Rat/Weight-drop	32°C	Contusion volume and edema	Hypothermia delayed edema formation but had no effect on contusion volume
1996 (55)	Rat/CCI	32°C, 35.4°C	Mortality	Both 32°C and 35.4°C reduced mortality rates
1996 (33)	Rat/CCI	32°C and/or Tirilizad	Axonal damage	Axonal damage reduced by either therapy but effects were not additive
1997 (40)	Rat/CCI	32°C	[14]PMN accumulation, [15]ICAM-1, [16]E-sel	Hypothermia reduced PMN accumulation

Date (Citation)	*Species/ Model*	*Temperature*	*Outcome parameter(s)*	*Key Finding(s)*
1997 (31)	Rat/CCI	30°C	Edema and cognitive outcome	Hypothermia reduced edema, no effect on cognitive deficits
1997 (56)	Rat/Weight-drop	32°C	EAA	Hypothermia did not reduce EAA levels
1997 (57)	Rat/Weight-drop	32°C	Nitrite and nitrate levels by microdialysis	Increase totally blocked by hypothermia
1998 (18)	Rat/CCI	32°C	Functional outcome and histopathology	Hypothermia reduced functional deficits; no effect on contusion volume or CA1/CA3 cell counts
1998 (26)	Dog/Epidural balloon	31°C	[17]ICP and herniation	ICP was reduced during cooling
1998 (58)	Rat/Freeze injury	32°C	[18]TUNEL and [19]DNA ladders	Hypothermia attenuated the increase in markers of apoptosis
1998 (48)	Rat/Impact acceleration	32°C	[20]APP levels	Hypothermia reduced APP positive axons
1999 (49)	Rat/FPI	30°C	[21]CBF and [22]CMRglu	After rewarming in the hypothermia group, CMR and CBF were mismatched at 3 h
1999 (45)	Rat/FPI	30°C	[23]cNOS and [24]iNOS	Hypothermia reduced the early increase in cNOS and the delayed increase in iNOS
1999 (37)	Rat/Impact acceleration with hypoxemia/ hypotension	30°C	Histopathology	Hypothermia provided almost complete protection
1999 (34)	Rat/Impact acceleration	32°C	Spectrin proteolysis	Marked reduction by hypothermia
2000 (41)	Rat/FPI	30°C	Myeloperoxidase	Hypothermia reduced the posttraumatic increase
2000 (24)	Rat/CCI with Hypoxemia	32°C	Functional outcome and histopathology	No effect of hypothermia
2000 (19)	Rat/CCI	32°C and/or [25]FGF	Functional outcome and histopathology	Hypothermia or FGF improved functional outcome but effects were not additive
2001 (21)	Rat/CCI	30°C	Functional outcome	Hypothermia was effective but results depended on level of injury severity
2001 (35)	Rat/Impact acceleration	32°C and/or [26]CyA	APP	Rewarming induced axonal injury which was attenuated by CyA
2001 (59)	Rat/Impact acceleration; propofol vs. isoflurane	33-34°C	CBF and ICP	Hypothermia with propofol reduced ICP most effectively

Date (Citation)	*Species/ Model*	*Temperature*	*Outcome parameter(s)*	*Key Finding(s)*
2001 (36)	Rat/Impact acceleration	32°C and/or FK506	APP	Rewarming induced axonal injury which was attenuated by FK506; implicates calcineurin as a target
2001 (25)	Rat/FPI with hypoxemia	30°C	Contusion volume	Demonstrated importance of rewarming rate
2001 (32)	Rat/CCI	30°C	Functional outcome and edema	60 min therapeutic window for beneficial effect of hypothermia on functional outcome and edema
2001 (60)	Rat/Impact acceleration	32°C	TUNEL and DNA ladders	Hypothermia attenuated TUNEL positivity in CA2 and CA3
2002 (20)	Rat/CCI	30-32°C and/or IL-10	Functional outcome, histopathology and PMN accumulation	Hypo improved functional outcome and CA3 neuron survival
2002 (44)	Rat/FPI	33°C	IL-1 mRNA	Hypo attenuated the posttraumatic increase
2002 (61)	Rat/FPI	30 or 33	Tissue hemoglobin	Hypo attenuated the posttraumatic increase
2003 (50)	Rat/CCI; fentanyl vs. isoflurane anesthesia	32°C,	Histopathology	Lesion volume was expanded by hypothermia on fentanyl anesthesia
2001 (28)	Mouse CCI	32°C	Histopathology, TUNEL, [27]PANT	Hypothermia reduced hippocampal neuronal death and DNA damage

[1]FPI= fluid percussion injury, [2]BBB = blood-brain barrier, [3]MAP2 = microtubule-associated protein-2, [3]CSF = cerebrospinal fluid, [4]ACh = acetylcholine, [5]EAA = excitatory amino acid, [6]ICP = intracranial pressure, [7]SOD = superoxide dismutase, [8]mRNA = messenger ribonucleic acid, [9]CCI = controlled cortical impact, [10]IL = interleukin, [11]NGF = nerve growth factor, [12]HSP-72 = heat-shock protein-72, [13]DHBA = dihydroxybenzoic acid, [14]PMN = polymorphonuclear leukocytes, [15]ICAM-1 = intercellular adhesion molecule-1, [16]E-sel = E-selectin, [17]ICP = intracranial pressure, [18]TUNEL = terminal deoxynucleotidyl transferase-mediated dUTP nick-end labeling, [19]DNA = deoxyribonucleic acid, [20]APP = amyloid precursor protein, [21]CBF= cerebral blood flow, [22]CMRglu = cerebral metabolic rate for glucose, [23]cNOS constitutive nitric oxide synthase, [24]iNOS = inducible nitric oxide synthase, [25]FGF = fibroblast growth factor, [26]CyA = cyclosporin-A, [27]PANT = DNA polymerase I-mediated biotin-dATP nick-translation.

Most of the experimental studies of moderate hypothermia in experimental TBI in the past decade were carried out in rats and a few in dogs and mice. Controlled cortical impact, fluid percussion, and impact acceleration models predominate in the rodent studies. Unlike the resurgence of studies of hypothermia in cardiac arrest models in the late 1980s, where mild hypothermia was the focus of investigation, the studies in experimental TBI have focused predominantly on moderate hypothermia. This is somewhat surprising, and the explanation for this fact is not completely clear. Although the studies in experimental TBI have focused on moderate hypothermia, recent randomized controlled trials (RCTs) of hypothermia in severe TBI have used either moderate or mild hypothermia, as discussed later.

Studies in rodent models of TBI have generally used a transient application (1-4 h) of moderate hypothermia (either 30 or 32°C). These studies have consistently demonstrated that moderate hypothermia attenuates functional deficits after injury (15-21). However, most studies suggest that the time window for the beneficial effect of hypothermia to improve functional outcome is short, between 30 and 60 min (16, 21). This suggests the importance of a direct effect of hypothermia on early secondary injury mechanisms, such as excitotoxicity or oxidative stress, rather than an effect on intracranial hypertension, which takes time to develop after injury. Studies of the effect of moderate hypothermia on histopathology have produced less consistent results than those assessing function. Experimental TBI produced by either controlled cortical impact or lateral fluid percussion generally produces a contusion with underlying hippocampal pathology (CA3, CA1, or hilar neuronal death). In contrast, the impact acceleration model produces minimal neuronal death but diffuse and focal (brainstem) axonal damage. Beneficial effects of hypothermia on contusion volume have been variable (17, 18, 22-26). More consistent effects on delayed hippocampal neuronal death in CA3 and/or CA1 have been reported (27, 28). This may result from the fact that at severe injury levels, necrosis within and around contusions is less amenable to therapies than delayed neuronal death in the hippocampus. In addition, penumbral cell death in CA3 or CA1 is associated with excitotoxicity, oxidative stress, and apoptosis, secondary injury mechanisms that may be specifically mitigated by hypothermia after TBI (discussed later).

A number of studies of experimental TBI have suggested beneficial effects of moderate hypothermia on brain swelling. Moderate hypothermia attenuates blood-brain barrier injury after either contusion or diffuse injury in rats (29, 30). Similarly, reductions in posttraumatic edema by moderate hypothermia have been reported in rodent models of cerebral contusion (22, 31, 32). The effect of moderate hypothermia on intracranial hypertension

has been studied most extensively in dogs subjected to local compression ischemia produced by epidural balloon inflation (23, 26). Moderate (31°C) but not mild (35°C) hypothermia dramatically reduced intracranial hypertension compared to controls when applied in this clinically relevant model of severe acute epidural hematoma. However, even when hypothermia was effective, secondary swelling and herniation during re-warming remained problematic.

Studies in multiple rodent models have shown that moderate hypothermia has favorable effects on traumatic axonal injury (33-36). This beneficial effect was demonstrated across multiple markers of axonal injury including assessment of spectrin degradation, amyloid precursor protein accumulation, and neurofilament compaction. Studies of the beneficial effects of moderate hypothermia on traumatic axonal injury have provided the most comprehensive assessment, to date, on the effect of rapid vs. slow re-warming in TBI and also have produced interesting results of combination therapies with hypothermia. Suchiro et al (29) reported more axonal damage when one hour of posttraumatic hypothermia was followed by rewarming over 20 min vs. 90 min. They also reported synergy between moderate hypothermia and either cyclosporin A or tacrolimus (FK 506), suggesting an avenue for combination therapy and implicating an important role for calcineurin in traumatic axonal damage.

SECONDARY INJURY MECHANISMS

Experimental studies have suggested variable effects of moderate hypothermia on TBI followed by a secondary hypotensive and/or hypoxemic insult (24, 25, 30, 37). Secondary insults after TBI often produce severe damage that may be refractory to even the most efficacious therapies. Patients with secondary insults have been generally excluded from the larger controlled clinical trials (38, 39).

Several secondary injury mechanisms are favorably influenced by moderate hypothermia in experimental TBI. It remains unclear, however, as to which of the multifaceted effects of hypothermia is the most important contributor to its therapeutic benefit. Moderate hypothermia has been shown to attenuate several components of the local inflammatory response to cerebral contusion, as evidenced by reductions in neutrophil accumulation (9, 40-42), interleukin-1 (IL-1) messenger ribonucleic acid (mRNA) upregulation (43, 44), and inducible nitric oxide synthase activity (45). Surprisingly, administration of the anti-inflammatory cytokine IL-10 negated the beneficial effects of hypothermia on functional and histological outcome after controlled cortical impact in rats (20). It may be risky to augment the

potent anti-inflammatory effects of moderate hypothermia after TBI. Moderate hypothermia can also attenuate posttraumatic excitotoxicity. Moderate hypothermia has been shown to reduce the posttraumatic increase in CSF levels of acetylcholine (16) and brain interstitial levels of glutamate and aspartate (46) in rats. However, a reduction of posttraumatic glutamate levels by moderate hypothermia has not been a consistent finding across experimental models (47, 48). Oxidative stress after TBI is linked to excitotoxicity along with a number of other secondary cascades. Moderate hypothermia has been shown to attenuate posttraumatic oxidative stress after fluid percussion in rats (46). This mirrors recent clinical findings that are discussed latter.

Not all of the studies of the effect of moderate hypothermia in experimental TBI have reported beneficial effects on outcome, and some clues may be derived from the negative studies. There has been little work on the effect of hypothermia on cerebral blood flow (CBF) after experimental TBI. Zhao (49) reported that moderate hypothermia (30°C for 3 h) reduced posttraumatic CBF at 3 h after rewarming in rats but failed to attenuate the increase cerebral metabolic rate for glucose (CMRglu), producing a mismatch between blood flow and metabolic demands. The increase in CMRglu after TBI is believed to result, in large part, from astrocyte-mediated uptake of the massive release in glutamate. Astrocyte uptake of glutamate is dependent entirely on glycolysis. Delayed excitotoxicity after rewarming may have occurred and this was not accompanied by a compensatory increase in CBF, leading to the observed mismatch after rewarming. One could envisage that during rapid rewarming, increases in metabolic demands from enhanced synaptic activity, stimulated glutamate re-uptake, and possibly subclinical status epilepticus, could be substantial and overwhelm the capacity of the injured cerebral circulation to keep pace and adequately vasodilate. Additional study of the effect of hypothermia and rewarming on CBF and CMR is warranted after experimental TBI.

Statler et al (50) recently compared transient moderate (32°C) hypothermia versus normothermia in rats anesthetized with fentanyl. Surprisingly, moderate hypothermia was associated with expansion of the lesion volume at 72 h after injury in rats anesthetized with fentanyl. Aspects of the stress response, such as plasma catecholamine levels were increased by hypothermia in the fentanyl anesthetized rats, suggesting the possibility of an exacerbated stress response by hypothermia. The beneficial effects of moderate hypothermia in most of the studies in Table 5-1 reflect its use in isoflurane-anesthetized rats. Clearly, more studies of the effect of hypothermia in experimental TBI are needed using clinically relevant anesthetic regimens.

EFFECT OF HYPOTHERMIA IN CLINICAL TBI

The resurgence in interest in mild hypothermia related to its beneficial effects in experimental cerebral ischemia and cardiac arrest sparked re-examination of hypothermia in experimental TBI. This rapidly lead to a re-examination of therapeutic hypothermia in clinical TBI, including several RCTs. Remarkably, over 25 clinical reports of the effect of therapeutic hypothermia on outcome, secondary injury mechanisms, or complications have been published since 1992 (Table 5-2). In contrast to the focus of experimental studies of TBI on moderate hypothermia, the studies of hypothermia in clinical TBI are equally mixed between those using mild or moderate levels; however, the vast majority of patients studied to date have been cooled to either 32 or 33°C.

Effect of hypothermia on ICP, physiology and secondary injury mechanisms

Two well-described studies carried out in 1993 evaluated the effect of moderate and mild hypothermia, respectively, on ICP and cerebrovascular physiology in adults after severe TBI. Marion et al (62) randomized 40 consecutive patients to either moderate hypothermia or normothermia and found that moderate hypothermia (maintained for 24 hours) reduced ICP, therapeutic intensity level (TIL), and CBF. The effects on ICP and CBF were limited to the initial 24 h after injury. $CMRO_2$ was not significantly altered. In a separate but concurrent study, Shiozaki et al (63) assessed ICP, cerebral perfusion pressure (CPP), CBF, $CMRO_2$ and mortality rate in 33 patients randomized to mild hypothermia versus normothermia. Hypothermia reduced posttraumatic intracranial hypertension, CBF, and $CMRO_2$, while improving CPP. The mortality rate from refractory intracranial hypertension was reduced from 12 of 17 to 5 of 16 patients in the normothermic vs. hypothermic groups, respectively, suggesting a powerful effect of mild hypothermia. Metz et al (64) reported similar effects on ICP in 10 patients with severe TBI, although a reduction in $CMRO_2$ was observed without an accompanying decrease in CBF with hypothermia. However, there was no concurrent control group. Shiozaki et at (65) reported on the effect of mild hypothermia (34°C) in a second prospective series of 62 patients with severe TBI and persistently raised ICP (> 20 mmHg) despite aggressive medical management. Mild hypothermia reduced ICP in 56.5% of these high-risk patients. In this series, mild hypothermia was most effective in patients with focal lesions. Tateishi et al [66] also evaluated patients with refractory intracranial hypertension after cerebral contusion, but used a titrated approach to both the depth and duration of

Table 5-2. Synopsis of selected clinical trials of therapeutic hypothermia in severe traumatic brain injury (TBI) since 1992

Date (Citation)	*Temp*	*Population*	*Outcome*	*Key finding*	*Comment*
1992 (87)	30-32°C	Prospective study of 21 patients undergoing elective craniotomy	Feasibility Complication	Two patients cooled to <32°C experienced ventricular arrhythmias or AV[1] block; no intracranial complications	
1993 (88)	32-33 °C vs. 37°C	Prospective RCT[2] of 46 patients with severe TBI	Phase II study	Trend toward improved GOS[3] in hypothermia group; Seizure incidence was reduced by hypothermia; no complications.	Cooling began within 6 h; 48 h duration
1993 (62)	32-33 °C vs. 37-38°C	Prospective RCT of 40 patients with severe TBI	[4]ICP, [5]TIL, [6]CBF, [7]$CMRO_2$, GOS	Moderate hypothermia reduced ICP, TIL, and CBF vs. normothermia	Target temperature reached in ~10 h; maintained for 24 h
1993 (63)	33.5-34.5 °C vs. normothermia	Prospective RCT in 33 patients with severe TBI	ICP, CPP, mortality, CBF, $CMRO_2$	Mild hypothermia reduced ICP, increased [8]CPP, reduced mortality rate. Mild hypothermia also decreased CBF and $CMRO_2$	CBF and $CMRO_2$ studied in a subgroup of 5 patients
1993 (89)	32-33 °C vs. normothermia	Prospective RCT in 36 patients with severe TBI	Delayed intracerebral hemorrhage; [9]PT, [10]PTT and platelet count	No differences between the two groups for any parameter	Cooling began within 6 h
1994 (68)	34.5-36°C	Case Report	Peritoneal cooling	Peritoneal cooling shown to be fast and effective in severe TBI	
1997 (64)	32.5-33°C; no control group	Prospective study of 10 patients with severe TBI	ICP; CBF; $CMRO_2$; [11]CMRlactate	Hypothermia reduced ICP and $CMRO_2$ and CMRlactate, but did not reduce CBF	Cooling began at a median of 16 h

Date (Citation)	*Temp*	*Population*	*Outcome*	*Key finding*	*Comment*
1997 (38)	32-33°C vs. normothermia	Prospective single-center RCT of 82 patients	3, 6, and 12 month GOS	Moderate hypothermia hastened neurologic recovery in patients GCS 5-7 and was associated with reductions in [12]CSF levels of glutamate and [13]IL-1	Target temperature achieved at median of 10 h; cooling for 24 h
1998 (66)	33-35°C; no control group	Prospective study; 9 patients with severe TBI	ICP	Mild hypothermia reduced ICP; Increased C-reactive protein and decreased platelet count	Hypothermia titrated to control ICP
1998 (73)	32-33°C vs. normothermia	Thirty-nine patients with severe TBI; Subgroup of an RCT	CSF levels of quinolinic acid, a macrophage marker	Hypothermia had no effect on CSF quinolinic acid levels	Suggests macrophage accumulation not attenuated by hypothermia
1998 (65)	34°C; no control group	Prospective study of 62 patients with severe TBI and persistent ICP > 20 mm Hg	ICP	Mild hypothermia reduced ICP in 56.5% of the patients whose ICP was > 20 mm Hg despite conventional treatment. Mild hypothermia more effective at controlling ICP in focal vs. diffuse swelling	
1999 (71)	32-33°C vs. normothermia	Prospective study of 23 patients with severe TBI	Jugular venous levels of IL-6	Moderate hypothermia reduced jugular venous levels of IL-6	Hypothermia at 4-9 d after injury
1999 (69)	34°C vs. normothermia	Prospective RCT of 16 patients with severe TBI, ICP<20 mm Hg	GOS; CSF [14]TNFα, IL-1β, IL-6, IL-8, IL-10	Most patients in both groups with good outcome; No reduction in CSF cytokines by hypothermia despite high levels of IL-6, IL-8 and IL-10	

Date (Citation)	*Temp*	*Population*	*Outcome*	*Key finding*	*Comment*
1999 (90)	30-33°C; no control group	Prospective study of 43 patients with severe TBI	Feasibility of prolonged hypothermia	Nosocomial pneumonia seen in 45% but death from sepsis rare (5%); no other complications	Hypothermia maintained a median of 8 d
2000 (84)	33-35°C; no control group	Prospective RCT of 87 patients with severe TBI	12 month GOS	GOS improved by hypothermia; Favorable outcome (GOS 4,5) was 46.5% in hypothermia vs. 27.27% in normothermia; Mortality rate was 25.58% in hypothermia vs. 45.45% in normothermia; ICP lower in the hypothermia group at d 7.	Duration of hypothermia 3-14 d; Hypothermia was stopped when ICP normalized
2000 (91)	32-33°C vs. normothermia	Prospective study of 26 patients with severe TBI	Jugular venous levels of thromboxane and 6-keto-$^{15}PGF_1\alpha$	Moderate hypothermia reduced the increase in jugular venous levels of thromboxane; no effect on 6-keto-$PGF_1\alpha$	
2001 (39)	33°C vs. normothermia	Prospective multi-center RCT of 392 patients	6 month GOS	Hypothermia failed to improve GOS; mortality rate was 28 and 27% in the hypothermia and normothermia groups, respectively; Fewer patients in the hypothermia group had elevated ICP; The hypothermia group had longer hospital stays and more complications	48 h treatment; mean time to target temperature was 8.4 h in hypothermia group

Date (Citation)	*Temp*	*Population*	*Outcome*	*Key finding*	*Comment*
2001 (92)	32-33°C vs. normothermia	Prospective study of 22 patients with severe TBI	Plasma phosphate concentration	Reduction in plasma phosphate concentration in hypothermia group that resolved with rewarming	
2001 (82)	33°C vs. normothermia	Further analysis of the multi-center RCT	Assessment of inter-center differences in a variety of parameters	Marginally different inter-center outcomes; wide differences in age, admission temperature, [16]MABP < 70 mmHg, and CPP < 50 mmHg between centers.	Suggests need for a detailed protocol for fluid and hemodynamic management for phase III trials in TBI
2002 (93)	34-36°C	Prospective study in 58 patients, 33 with persistent ICP >20 mmHg treated with hypothermia	Brain temperature monitored (multi-parameter probe)	Brain temperature monitoring is feasible; difference between brain and rectal temperature was directly correlated with outcome	Suggests that low brain vs. rectal temperature is predictive of poor outcome
2002 (94)	34-36°C	Prospective study in 58 patients, 33 with persistent ICP >20 mmHg treated with hypothermia	Brain tissue pO_2, pCO_2, pH; brain interstitial levels of glutamate and lactate	Mild hypothermia reduced brain tissue pO_2 and pCO_2 and increased brain tissue pH. Patients with spontaneous hypothermia on admission had high levels of glutamate and lactate	
2002 (83)	33°C vs. normothermia	Further analysis of the multi-center RCT	Assessment of fluid balance during the 96 h after randomization	Fluid balance varied from −10 L to +20L. A fluid balance lower than −594 mL was associated with poor outcome	Variability in fluid balance for patients with isolated TBI; suggests dehydration therapy in some patients

Date (Citation)	*Temp*	*Population*	*Outcome*	*Key finding*	*Comment*
2002 (95)	Not applicable	Meta-analysis of the studies carried up to and including 2001	GOS, ICP, complications	No beneficial effect of hypothermia on any parameter, the only complication significantly influenced was mild prolongation of PTT	Seven studies included; 368 of the 668 patients were part of one study (52)
2002 (96)	34 °C vs. normothermia	Thirty patients with severe TBI divided into two groups	GOS and ICP	GOS did not differ between treatment groups but ICP was reduced by hypothermia	72 h of cooling; European Brain Injury Consortium protocol
2002 (79)	33-35°C vs. normothermia	Thirty five severe TBI patients were treated vs. separate controls	GOS	GOS better in hypothermia group vs. normothermic controls. Age > 50 years was associated with poor outcome	
2002 (98)	33°C vs. normothermia	Further analysis of the multi-center RCT (52)	Assessment of the impact of hypothermia on admission	Hypothermia on admission associated with improved outcome	Suggests possible benefit of early posttraumatic hypothermia
2003 (99)	33°C; no control group	Thirty one severe TBI patients all treated with hypothermia	ICP	ICP decreased at 35-36°C, but no differences seen at temperatures below 35°C; CPP also peaked at 35-36°C; CPP decreased below 35°C	Suggests that mild hypothermia can reduce ICP in some cases
2003 (74)	RCT of 32°C vs. normothermia	Prospective study of 28 infants and children with severe TBI	CSF levels of markers of oxidative stress	Reduction in both lipid peroxidation (F_2-isoprostane) and loss of endogenous antioxidants by moderate hypothermia	

Date (Citation)	*Temp*	*Population*	*Outcome*	*Key finding*	*Comment*
2003 (72)	RCT of 32°C vs. normothermia	Prospective study of 28 infants and children with severe TBI	CSF levels of IL-6, IL-8	Marked increase in CSF levels of IL-6 and IL-8 after injury but no difference in hypothermia vs. normothermia	Selective effects of moderate hypothermia on biochemical cascades
2003 (70)	32-33°C vs. normothermia	Sixty-eight adults; subset of patients from the multi-center RCT (52)	CSF levels of glutamate, and F_2-isoprostane	Hypothermia reduced both glutamate and F_2-isoprostane; the magnitude of the reduction was greater for glutamate than isoprostane	
2003 (85)	Not applicable	Meta-analysis of 12 trials including 1069 adults with severe TBI	Mortality and neurological outcome	Overall benefit of moderate or mild hypothermia (32-33°C); 19% relative reduction in the risk of death and a 22% relative reduction in the risk of poor neurological outcome vs. normothermia.	Favorable effects of cooling for 24-48 h, or longer, target of 32-33°C, and rewarming duration ≤24 h

[1]AV = atrioventricular, [2]RCT= randomized controlled trial, [3]GOS = Glasgow outcome scale, [4]ICP = intracranial pressure, [5]TIL = therapeutic intensity level, [6]CBF = cerebral blood flow, [7]$CMRO_2$ = cerebral metabolic rate for oxygen, [8]CPP = cerebral perfusion pressure, [9]PT = prothrombin time, [10]PTT = partial thromboplastin time, [11]CMRlactate = cerebral metabolic rate for lactate, [12]CSF = cerebrospinal fluid, [13]IL = interleukin, [14]TNFα = tumor necrosis factor alpha, [15]PGF = prostaglandin-F, [16]MABP = mean arterial blood pressure,

hypothermia. In his series, ICP was controlled in 8 of 9 patients using relatively mild hypothermia—with a temperature range between 33 and 35°C. The mean duration of cooling required to control ICP was 68 h, and some patients were cooled for 4 days. One patient died of septicemia; platelet counts decreased to less than 100,000/μL in 5 of the 9 patients by day 4. A number of other authors (Table 5-2) have reported reductions of ICP in patients with severe TBI treated with either mild or moderate hypothermia, and this finding was confirmed even in the multi-center trial carried out by Clifton (39). Studies in an animal model of TBI suggest that moderate hypothermia is necessary to control intracranial hypertension (23, 26). However, clinical studies indicate that mild hypothermia is often

successful at controlling ICP, even in many cases refractory to medical management. Recently, Tokutomi et al (67) confirmed the efficacy of mild hypothermia by carefully evaluating the effect of temperature level on ICP during cooling to 33°C in 31 adults with severe TBI. Surprisingly, the decrease in ICP and improvement in CPP was greatest at 35.5°C.

In all of these studies hypothermia was induced by surface methods with or without gastric lavage. In a report on a single case, Cancio et al (68) described rapid cooling using peritoneal lavage in a patient with severe TBI. Inotropic and/or pressor agents were required to support hemodynamics, but complications of hypothermia were generally reported as manageable.

The effects of hypothermia on a number of secondary injury mechanisms after severe TBI, have been assessed in CSF, jugular venous blood, or brain interstitial fluid. Marion et al (38), in a small subset of patients from his single center RCT, reported that moderate hypothermia attenuated the increase in CSF levels of glutamate, suggesting a key beneficial effect of moderate hypothermia on excitotoxicity. In contrast, Shiozaki et al (69) serially assessed CSF levels of glutamate, aspartate, and glycine in 16 patients randomized to mild hypothermia (34°C) vs. normothermia. No differences between treatment groups in any of excitatory amino acids was seen in hypothermic versus normothermic groups; however, glutamate levels were only modestly increased on admission, and the other excitatory amino acids were not increased. Recently, Wagner et al (70) reported a marked reduction of CSF glutamate by moderate hypothermia vs. normothermia in a large study of patients (n = 68) with severe TBI.

Marion et al (38) reported a reduction in CSF levels of IL-1β by moderate hypothermia—although the increases in IL-1β were modest in magnitude. Nevertheless, this suggested a beneficial effect of hypothermia on posttraumatic inflammation. Aibiki et al (71) measured levels of the cytokine IL-6 in jugular venous samples from 23 adults randomized to moderate hypothermia (32-33°C) or normothermia after severe TBI. Hypothermia attenuated the increase in IL-6 after injury. In contrast, Shiozaki et al (69) serially assessed CSF levels of a battery of cytokines (tumor necrosis factor [TNF]α, IL-1β, IL-6, IL-8, and IL-10) in his previously described study of 16 patients with TBI (but without elevated ICP) randomized to mild (34°C) vs. normothermia. No differences between treatment groups were also seen for the cytokines, despite the fact that CSF levels of some cytokines such as IL-6 and IL-8 were dramatically increased after injury. Similar findings were recently reported by Shore et al (72)—who reported no difference in CSF levels of either IL-6 or IL-8 after severe TBI in infants and children treated with moderate hypothermia vs. normothermia. Sinz et al (73) studied the effect of moderate hypothermia vs. normothermia on CSF levels of the macrophage marker quinolinic acid

in adults with severe TBI. CSF quinolinic acid levels did not differ between treatment groups—suggesting that macrophage accumulation was neither attenuated nor delayed by the use of moderate therapeutic hypothermia. Thus, except for IL-1β in CSF and IL-6 in jugular venous blood, increases in cytokines after severe TBI are not consistently attenuated by mild or moderate hypothermia.

Another dramatic effect of moderate hypothermia on the secondary injury cascade was recently reported by Bayir et al (74) and Wagner et al(70). Bayir et al (74) reported that moderate hypothermia (vs. normothermia) markedly attenuated both lipid peroxidation and consumption of endogenous antioxidants after severe TBI in infants and children. Wagner et al (70) similarly reported that moderate hypothermia attenuated both glutamate and F2-isoprostane levels in adults after severe TBI in adults. The effect of hypothermia was greater on glutamate levels than on F2-isoprostane levels. Since excitotoxicity and oxidative stress are linked in TBI, this suggests that inhibition of excitotoxicity likely contributes substantially to the reduction in oxidative stress by hypothermia. In addition, CSF levels of markers of lipid peroxidation are lower in women than in men after TBI; and the reduction of posttraumatic oxidative stress by moderate hypothermia in adults is easier to demonstrate in males than in females (75). Much additional work is needed to define the secondary injury cascades that are favorably influenced by mild or moderate hypothermia; however, a picture is emerging that mild and moderate hypothermia do not indiscriminately slow all biochemical reactions in the secondary injury cascade, rather they appear to have selective effects, particularly on excitotoxicity and oxidative stress.

Finally, hypothermia is a unique modulator of protein synthesis. Although it depresses overall protein synthesis, it selectively increases the synthesis of stress proteins via cold shock stress (76). Cold stress protein expression is affected by the depth and duration of hypothermia as well as the rate of rewarming. Hypothetically, these parameters could be intentionally manipulated to selectively increase the protein expression of neuroprotective protein effectors. In support of this approach, several studies in experimental brain injury have suggested that mild or moderate hypothermia selectively stimulates production of neuroprotective factors (rather than inhibits deleterious pathways). Brain-derived neurotrophic factor (BDNF) has been suggested to be an example of a neuroprotective molecule stimulated by mild hypothermia after experimental brain ischemia/reperfusion (77). However, recent clinical studies have not demonstrated parallel increases in BDNF in CSF samples from patients randomized to hypothermia vs. normothermia after severe TBI (72). Further study is needed on this interesting hypothesis.

CONCLUSIONS

There is strong evidence that hypothermia is effective in preventing the development of raised ICP and/or controlling refractory intracranial hypertension after TBI. Even mild levels can often achieve this goal. For optimal control of intracranial hypertension, hypothermia should probably be titrated, facilitating application of mild rather than moderate hypothermia whenever possible, and avoiding prolonged application. The success rates for the effect of hypothermia in the treatment of refractory intracranial hypertension appear similar to those reported for other therapies such as barbiturates (78). Mild and moderate hypothermia tend to reduce CBF and $CMRO_2$, but these effects are not consistently observed across studies.

After severe TBI, application of mild or moderate therapeutic hypothermia may have cerebral resuscitative effects that occur independent of a reduction of ICP. Recent clinical studies in cardiac arrest (79-81) certainly support a direct neuroresuscitative effect. Although this is likely in TBI, based on the single center trials presented in this review and the extensive data in experimental models, much of the neurotrauma community remains unconvinced. In addition, the optimal temperature, duration, and other details for its use in this manner remain to be determined. Although the multi-center RCT of Clifton et al (39) failed, the variability in the approach to maintaining a target CPP in that study (fluids vs. pressors, etc) was a recognized problem (82, 83). This problem was avoided in two fairly large single center RCTs (38, 84). Several clinical trials in severe TBI are ongoing including pediatric trials and a new adult trial, although it is likely these will all test moderate hypothermia. A recent meta-analysis demonstrated a positive overall effect of therapeutic hypothermia in severe TBI in adults (85). Hopefully, mild hypothermia will receive further clinical investigation in severe TBI, perhaps including rapid induction of cooling via intravenous administration of cold saline (86). Finally, considerable laboratory evidence supports the deleterious effects of hyperthermia after severe TBI; fever should be rigorously avoided.

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Therapeutic Hypothermia

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Learning Objectives: 1) to familiarize the reader with contemporary studies on the application of resuscitative hypothermia in the treatment of traumatic brain injury and hemorrhagic shock, 2) to describe the potential mechanisms for the beneficial effects of hypothermia in these settings, 3) to present some recent findings from both laboratory and clinical studies of resuscitative hypothermia conducted at the University of Pittsburgh, 4) to discuss possible side effects and limitations of the application of therapeutic hypothermia, and 5) to discuss future directions for novel applications of hypothermia.

Abstract

There have been some exciting advancements in the field of therapeutic hypothermia during the past 7 years. Studies have shown the beneficial effects of mild hypothermia after ventricular fibrillation cardiac arrest and its use has been recommended by two leading medical groups. Ongoing work in the areas of the therapeutic efficacy of mild cooling and methods for rapid reduction and use during field resuscitation have been presented. The importance of mild cooling during CPR—a subject of intense investigation by the late Dr. Peter Safar near the end of his career—is beginning to become evident in laboratory studies. Mild hypothermia is also under additional investigation for use as treatment of severe traumatic brain injury. Further work is needed in this area in both children and adults because of controversial findings in the recent adult multicenter clinical trial. There also have been developments in the area of the potential applications of hypothermia in resuscitation from hemorrhagic shock. The studies of this controversial use are still in the experimental stage. Finally, we discuss a novel approach to treatment of exsanguination cardiac arrest by application of suspended animation with delayed resuscitation.

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In 1997, our group at the Safar Center for Resuscitation Research consulted on an article for the ITACCS-sponsored monograph on hypothermia in trauma that was entitled *Therapeutic Hypothermia After Traumatic Brain Injury or Hemorrhagic Shock: From Mild Cooling to Suspended Animation.*[1] Having been asked to update that article for *TraumaCare* in 2004, we take a look back and a glimpse forward on the topic of therapeutic hypothermia in the collective field of resuscitation medicine.

From 1997 to 2004

Unquestionably, the most exciting and important development in the field of therapeutic hypothermia for resuscitation medicine came in February 2002. In that month, two separate studies were reported in the *New England Journal of Medicine* demonstrating beneficial effects of mild hypothermia (~33°C) after ventricular fibrillation (VF) cardiac arrest (CA) in adults.[2-4] Sterz and his multicenter group in Europe[2] and Bernard et al[3] in Australia reported significant beneficial effects on outcome when hypothermia was initiated after restoration of spontaneous circulation. In the study by Sterz and colleagues,[2] to prevent one unfavorable outcome, six patients would need to be treated with hypothermia. Cooling was continued for either 12 hours in the study by Sterz and colleagues[2] or 24 hours in the study by Bernard and colleagues.[3] Even more surprising to our group in Pittsburgh was the fact that cooling was effective even though the time to target temperature was about 12 hours in the study by Sterz and colleagues.[2] This suggests benefit of hypothermia after cardiac arrest even with delayed application. One mechanism that may be involved is the ability of mild cooling to block delayed neuronal death, which is likely to develop as part of an activated apoptosis cascade after CNS injury.[5-7] Blockade of the release of the key initiator of the mitochondrial intrinsic pathway of apoptosis—cyctochrome C—by mild hypothermia was recently shown in experimental brain ischemia.[7] Of interest, successful delayed application of mild hypothermia has been shown in experimental animal models.[8] These two clinical studies prompted a recent Level I recommendation of the American Heart Association (AHA) and the International Liaison Committee on Resuscitation (ILCOR) for the use of mild hypothermia after VFCA in adults.[9]

It is, however, widely recognized that therapeutic hypothermia is most efficacious when applied either before or early after CNS insults. In this regard, there have been two important studies that we believe will further expand the therapeutic efficacy of mild cooling. In 2003, in a study of 22 adults, Bernard et al[10] reported that 30 mL/kg bolus over 30 min of an ice cold (4°C) lactated Ringer's solution is safe and reduces care core temperature by ~2°C when administered after establishing stable restoration of spontaneous circulation (ROSC) in CA victims. This is a simple, inexpensive, and very feasible approach to rapid induction of mild hypothermia. Ambulances should develop systems to have several liters of ice cold fluid readily available. More recently, in an experimental laboratory model of CA in dogs simulating field resuscitation, Nozari et al[11] carried this concept further and

reported that mild hypothermia was powerfully effective in improving survival and outcome when initiated during protracted CPR and ACLS. Clinical trials of the use of mild cooling during CPR should be pursued. Of note, that work was one of several that CPR pioneer Dr. Peter Safar believed to be of special importance to the optimization of hypothermia, during his investigation in the final 15 months of his life.[12]

Traumatic Brain Injury

Remarkably, in 1997 there was little hope that hypothermia would be effective in CA, but much optimism that it would become standard of care in the setting of severe traumatic brain injury (TBI). That optimism was based on the study of the beneficial effect of moderate hypothermia (32°C) on outcome published by Marion et al.[13] This study followed a long series of positive reports on the effect of moderate hypothermia in experimental TBI. However, Clifton et al,[14] in 2001, reported on the failure of moderate hypothermia (32°C) in 392 patients in a study that staggered the momentum behind the application of this therapy in CNS injury. Mild and moderate hypothermia had been shown consistently to have the most powerful beneficial effect on outcome of any therapy in experimental TBI; why had it failed? And now, in light of the positive trials in CA, why would therapeutic hypothermia be effective in CA but not in severe TBI? This topic was recently reviewed.[15] One of the answers may lie in the mire of the challenges of carrying out a multicenter study of this magnitude and in the complex therapeutic setting of severe TBI. Issues such as differences in the approaches taken between centers to achieve the target cerebral perfusion pressure and intracranial pressure (i.e., fluid versus pressor) along with the fact that a number of patients in both groups presented with hypothermia on admission have been discussed in separate reports.[16,17] Another intriguing possibility is the fact that unlike TBI, there is generally no application of other brain-oriented therapies (ICP monitoring, mannitol, hypertonic saline) in CA. Thus, hypothermia was compared with a number of other brain-oriented therapies in TBI that may already be mitigating most or all of the secondary damage that is therapeutically manipulable by cooling.

The optimal temperature, duration, and rate of rewarming could also be factors that need to be defined for successful application of therapeutic hypothermia. It is interesting that in CA mild hypothermia (33–36°C) is generally used, while in TBI moderate hypothermia (32°C) is generally the target. Recently, Tokutomi et al[18] reported that extremely mild hypothermia (35.5°C) may be optimal in clinical TBI. Similarly, rapid rewarming may be particularly harmful to traumatically injured brains.[19] Finally, sex may be an important factor in determining the efficacy of hypothermia in TBI. Bayır et al[20] recently reported that lipid peroxidation (assessed by CSF levels of F_2-isoprostane) after severe TBI in adults was markedly increased on day 1 in male patients, but not in female patients. Hypothermia was only able to affect this mechanism in men. Further clinical and laboratory study of hypothermia in TBI is needed.

An additional area of investigation that is ongoing in Pittsburgh in the area of hypothermia in TBI is the multicenter safety and feasibility study of infants and children by P. David Adelson and his group.[21] This work actually includes two studies: a multicenter trial in cases in which the time of injury is less than 6 hours before enrollment and a second trial that includes children presenting with secondary deterioration and child abuse victims where the time of injury is not defined. As these studies are being carried out by Dr. Adelson and colleagues, our investigators at the Safar Center have performed a comprehensive assessment of the effect of therapeutic hypothermia (32–33°C) applied for 48 hours on CSF biochemistry in these infants and children. Our data support a powerful beneficial effect of hypothermia on a battery of markers of oxidative injury.[20] However, the effect of hypothermia appears to be selective since we did not observe reductions in the posttrauma increase in a number of other markers of secondary damage.[22] As additional data emerge from this trial, we hope we will be able to better understand and tailor the use of hypothermia in pediatric and adult TBI.

Hypothermia in Hemorrhagic Shock

Since publication of our review in 1997, there have been a number of developments in the area of the potential applications of hypothermia in resuscitation from hemorrhagic shock. These studies have been exclusively carried out in experimental models since the use of mild cooling during traumatic hemorrhagic shock is more controversial than its use in cerebral resuscitation and preservation. Tisherman and co-investigators at the Safar Center[23-28] have led the way in the investigation of this potential use of mild hypothermia.

In a series of studies in rats, mild cooling during shock was found to increase survival in both uncontrolled and pressure-controlled models.[23-28] In this setting, cooling appears to confer a systemic benefit since local gut cooling was insufficient to confer protection.[27] One possible mechanism for the benefit of hypothermia in this setting is shown by the work of Weisser et al.[29] They reported that mild cooling improves myocardial contractility during shock. Finally, in a recent study, Wu et al[28] demonstrated a beneficial effect of mild IV cooling on survival in a pig model of trauma and hemorrhagic shock. A surprising finding in that study was the fact that rapid cooling with IV iced saline was not as effective as somewhat slower controlled cooling with room temperature IV fluids. Although additional investigation of this controversial but interesting application of mild hypothermia is needed in large-animal models of hemorrhagic shock, clinical feasibility trials could be initiated.

Suspended Animation with Delayed Resuscitation for Exsanguination Cardiac Arrest

Finally, our group has been intensely studying a very novel approach to the treatment of victims of exsanguination CA. This work has been recently reviewed,[30] but has been developed as part of a novel approach to the high field mortality from rapid exsanguination seen in combat casualties. Using an aortic flush of iced (~ 2°C) saline initiated at 2 minutes after established exsanguination CA in a dog model that includes 72 hours of contemporary ICU care, preservation times of up to 2 hours have been achieved with normal long-term outcome.[31] A brain temperature of ~10°C is used with this approach to achieve good outcome for arrest times beyond 60 minutes. The effect of profound hypothermic preservation has been also found to be efficacious even in the setting of exsanguination CA with superimposed tissue trauma (splenic laceration and laparotomy); however, the addition of postresuscitation plasma exchange was necessary for optimal outcome.[32] In current studies in our laboratory we are testing if this suspended animation approach is still successful in

achieving normal outcome if prolonged hemorrhagic shock (1.5–2.5 hours) precedes exsanguination CA. The beneficial effects of this extremely novel approach to trauma resuscitation are remarkable. The first clinical application of suspended animation with delayed resuscitation is being considered in the civilian of exsanguination CA from penetrating trauma.[30]

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Therapeutic Hypothermia in Resuscitation: The Safar Vision

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Much has been written about the incredible life of Peter Safar (Fig. 1), inventor of modern-day CPR, pioneer in anesthesiology, critical care medicine, emergency medicine, and disaster reanimatology, and humanist and mentor to countless clinicians, scientists, and students. For any of you who are interested in learning more about this incredible man, a comprehensive review of Peter's contributions to resuscitation can be found in a two-part series written by Peter Baskett and published in the journal *Resuscitation*.[1,2] Peter Safar's autobiography is also available through the Wood Library-Museum of Anesthesiology, and provides remarkable detail on both his academic and personal endeavors.[3] Finally, Drs. Patrick Kochanek, Ake Grenvik, and John Schaefer and Ms. Fran Mistrick assembled a Festschrift in honor of Dr. Safar, published in February 2004, as an entire freestanding supplement to the journal *Critical Care Medicine*.[4] That document provides a pictorial synopsis of his career, and quotes from over 30 individuals on their recollections of Peter Safar.

In light of the focus of this issue of *TraumaCare* on the use of therapeutic hypothermia in resuscitation and trauma, we thought that it would be worthwhile to write a short tribute to Peter specifically focused on some of his thoughts about the development and application of therapeutic hypothermia across the spectrum of resuscitation medicine.

Safar's Definitions of Hypothermia and Its Use in Resuscitation

On the topic of therapeutic hypothermia in resuscitation, Peter Safar would always begin by pointing out two issues that he believed were critical about this area of study, namely, accurate and consistent terminology concerning the depth of hypothermia and the situation surrounding its use. He believed that hypothermia should be categorized into mild (34–36°C), moderate (27–33°C), deep (15–26°C), profound (10–14°C), and ultraprofound (<10°C), and that the consistent use of this terminology was important since different mechanisms are affected in each of these temperature ranges. Similarly, he emphasized the importance of categorizing the use of hypothermia (and other therapies in the field of resuscitation medicine) as used for protection (applied before the insult), preservation (applied during the insult), or resuscitation (applied after the insult). These were part of the language of resuscitation, and he emphasized that we must speak it consistently to optimally communicate in our field. He disliked the term "neuroprotection" that is so often used by scientists and clinicians when discussing therapies that might be used in cerebral resuscitation after traumatic brain injury or cardiopulmonary arrest. As one can infer from the foregoing statements, these would be resuscitative rather than protective or preservative therapies.

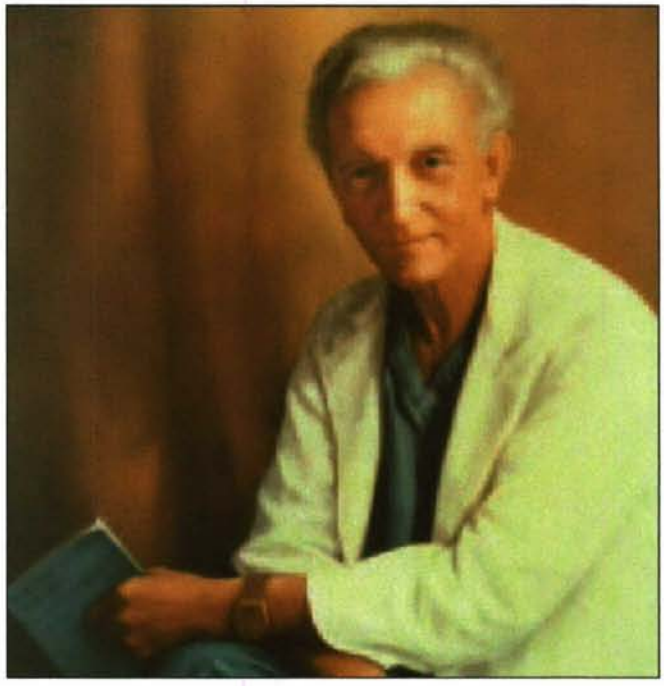

Figure 1. Peter Safar, MD (See cover for portrait details)

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Peter Safar and Resuscitative Hypothermia in the 1960s to 1980s

Peter Safar was intimately involved in the use of therapeutic hypothermia in the 1960s in the treatment of patients across a broad spectrum of disorders during the birth of modern-day neurointensive care. He was heavily influenced by the work of Dr. Hugh Rosomoff in the Department of Neurological Surgery at the University of Pittsburgh, who was one of the pioneers in the investigation and application of therapeutic hypothermia in the 1950s and 1960s.[5-7] Peter Safar came to Pittsburgh in 1961 and always respected Rosomoff's work. In subsequent years, he fondly discussed the interaction between anesthesiology and neurological surgery in the use of hypothermia in patients with cerebral swelling. Safar often described the importance of titration of the hypothermia used in these patients. For example, he often indicated that in the 1960s, they routinely applied moderate hypothermia to patients with intracranial hypertension and severe traumatic brain injury, and would reinstate it if a secondary rise in intracranial hypertension occurred during rewarming. In traumatic brain injury, he did not believe that a single value for temperature control made sense, rather, that the depth of hypothermia should be continuously titrated to optimize its effect on a physiologically relevant bedside parameter, namely, intracranial pressure.

Safar also learned a great deal about therapeutic

hypothermia in the early 1960s from the work of others outside Pittsburgh. For example, he was always intrigued by the work of Dr. Robert White in Cleveland, Ohio, who performed a number of pioneering studies of the use of hypothermia to preserve the isolated dog brain.[8,9] Similarly, he also discussed the early use of hypothermia by Lundberg and co-workers[10] in Lund, Sweden, and early use of spinal cord cooling by Albin et al.[11] However, in retrospect, Peter Safar recognized that there was inadequate information available about how to optimize the application of hypothermia in that early era and that the side effects seen with the use of moderate hypothermia for prolonged periods (particularly pulmonary infection and sepsis) gradually led to its abandonment in clinical use.

In reviewing the collected works of Peter Safar, the earliest description of his thoughts on the use of therapeutic hypothermia are provided in an amazing article written by him and published in a 1964 issue of the *Journal of the Iowa Medical Society.*[12] Peter Safar's "ABCs" (and beyond) of resuscitation in the early 1960s are described in this article—and of course Peter Safar was not satisfied with just ABC. He provided the resuscitator an entire alphabet of interventions for the victim in cardiac arrest. Prophetically, this describes the letter "H" in his resuscitation alphabet as the application of therapeutic hypothermia. This description is not all that far from what recently received a Level I endorsement from the International Liaison Committee on Resuscitation (ILCOR)[13] and the American Heart Association (AHA).[14] The figure outlining Peter's ABCs from 1964 and the use of hypothermia is shown in Figure 2.

Figure 2. Figure from a 1964 publication by Peter Safar in the *Journal of the Iowa Medical Society* describing the Safar ABCs and beyond of resuscitation. Over 40 years ago Peter Safar included the postresuscitation induction of hypothermia (see arrow) in victims of cardiopulmonary arrest. This concept was recently endorsed into standard of care (see text for details).

In the laboratory, one of the earliest documentations of therapeutic hypothermia in the collected works of Dr. Safar is an interesting report by Gisvold et al[15] in 1984 that described the use of a multifaceted therapeutic approach to cerebral resuscitation in an experimental model of complete global cerebral ischemia in monkeys. The approach used hemodilution, transient hypertension, pentobarbital, dexamethasone, and 6 hours of hypothermia, which significantly improved intact survival in 7 of 10 versus 2 of 9 controls. The concept and testing of a multifaceted approach to cerebral resuscitation, including hypothermia, was certainly ahead of its time, and contributed to the rejuvenation in the use of hypothermia that was also stimulated by the work of Busto et al,[16] who showed that very small reductions in brain temperature improved outcome in experimental cerebral ischemia in rats. The resurgence in interest in hypothermia that followed is recent history, with which you all are surely familiar.

In the last 20 years, Peter Safar focused on the use of hypothermia since it was the only therapy that he found to consistently demonstrate a "breakthrough" effect in his experimental models. In 2002, two large clinical trials demonstrated the efficacy of mild hypothermia after ventricular fibrillation (VF) cardiopulmonary arrest in humans[17-19] and, as previously described, this has now been recommended for clinical use by the key endorsing societies worldwide.[13,14]

On the day that the ILCOR and AHA guidelines were published, endorsing mild hypothermia after VF cardiac arrest in adults, I (PK) went into Peter Safar's office to share with him this exciting news. In typical Safar fashion he stated, "What took them so long?" When a therapy was shown to be effective—based on sound experimental evidence in large-animal studies that included clinically relevant long-term outcome and intensive care unit (ICU) care, and that accurately modeled the clinical condition—Peter Safar believed that randomized clinical trials were needed only to show feasibility. Peter Safar was not convinced that randomized clinical trials (RCTs) were very helpful in the difficult setting of resuscitation medicine, where it is challenging to control any of the key physiological parameters or underlying disorders. He believed that if a therapy was shown to be feasible and safe in clinical trials, and effective in relevant laboratory models, we were depriving patients of a valuable therapy that may never be able to be proven effective in the morass of an RCT. Fortunately, mild hypothermia was powerful enough to demonstrate a beneficial effect in two RCTs. Based on Peter's understanding of the problems that we face in resuscitation research, other agents able to demonstrate a benefit in an RCT are likely to need to possess similar breakthrough effects in laboratory studies.

Peter Safar and Resuscitative Hypothermia: Recent Investigation

Peter Safar also carried out a considerable body of work in the last 20 years to support the use of hypothermia on three additional fronts that are relevant to readership of *TraumaCare*. First, he worked closely with trauma surgeon and critical care physician Samuel Tisherman on the use of mild hypothermia to prolong the "golden hour" of shock. That work is in a highly controversial area because retrospective clinical studies associate exposure/secondary hypothermia with increased mortality rate. However, the studies of Safar and Tisherman in this area represent a substantial series of experiments in rodent and pig models of hemorrhagic shock, demonstrating that mild or moderate hypothermia can delay the time to exsanguination

cardiopulmonary arrest in this condition.[20-25] Second, he developed, after discussions with Colonel Ronald Bellamy of the United States Army, a novel approach to the resuscitation of victims of exsanguination cardiopulmonary arrest. He proposed inducing a brief (several-hour) state of suspended animation using an aortic flush of a cold preservative solution that could buy time for transport and surgical repair, which could be followed by delayed resuscitation using cardiopulmonary bypass.[26-29] We at the Safar Center have been fortunate to participate in this landmark project, which, to date, has been able to successfully achieve good outcome in dogs after an exsanguination cardiopulmonary arrest of 2 hours' duration using profound hypothermia (10°C).[30] It will be interesting to see over the years that follow if clinical trials are carried out in either of these two extremely novel areas of research.

Finally, Peter Safar and co-workers also carried out some of the only contemporary work on the application of therapeutic hypothermia to the treatment of traumatic brain injury using large-animal models. Despite the considerable number of studies in contemporary models of experimental traumatic brain injury in rodents, few studies supported its use in large-animal models, with clinically relevant long-term outcome, ICU care, and intracranial pressure (ICP) monitoring and control. Peter's group published two such papers on the efficacy of moderate hypothermia in a canine model of epidural hematoma.[31,32] He believed that it was essential to test resuscitation-related therapies in large-animal models that mimicked the clinical condition as closely as possible.[33]

Some of Peter Safar's final experimental work demonstrated the incredible vision that he possessed, the value that he placed on translational studies, and his obsession that one cannot be satisfied until a therapy is optimized—and used. With the acceptance of mild hypothermia as a therapy after successful restoration of spontaneous circulation (ROSC), Peter questioned why we were waiting to apply this therapy "after" ROSC. Indeed, applied during advanced cardiac life support in a model of prolonged experimental VF in dogs, Nozari et al[34] demonstrated that mild hypothermia was dramatically beneficial to both cerebral and myocardial outcome. Mild hypothermia applied during resuscitation, in a preservative rather than resuscitative manner, is a therapy that deserves to be tested in clinical trials.

Peter Safar was also interested in understanding the mechanism(s) underlying the beneficial effects of therapeutic hypothermia in protection, preservation, and resuscitation. Some of his last work was done in collaboration with Drs. Larry Jenkins and Mandeep Chadha on the use of proteomics to determine if profound hypothermia was preventing proteolysis during prolonged ischemia.[35] However, it was the study of the effect of therapies on outcome in clinically relevant experimental models that Dr. Safar believed was the most important.

Peter Safar's Overall Vision on the Potential of Therapeutic Hypothermia

Peter's vision on hypothermia in resuscitation was that it was the most effective agent that was currently available in resuscitation medicine—and that it had important potential applications in at least 10 different disease processes, including 1) VF, asphyxial, and exsanguination cardiopulmonary arrest; 2) traumatic brain injury; 3) stroke; 4) acute myocardial infarction; 5) elective surgical procedures; 6) refractory status epilepticus; 7) septic shock; 8) spinal cord injury; 9) hemorrhagic shock; and 10) possibly even septic shock. He also believed that rigorous temperature control with prevention of fever in neurointensive care was logical and should be implemented. He believed that clinical feasibility and safety studies should be performed in each of these settings, followed by clinical application.

Conclusions

In 2003, Drs. Kochanek and Safar wrote an editorial on the use of therapeutic hypothermia in traumatic brain injury that was published in the *Journal of the American Medical Association*.[36] Although other articles with Dr. Safar as co-author will continue to appear over time in the literature, since many works that he sparked are still in progress, that article represents the final paper that Peter Safar worked on before his death. The need to titrate, optimize, and better understand the mechanistic effects of hypothermia while we use it to improve outcome in our patients resonates from his final work.

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The Physiology of Mammalian Temperature Homeostasis

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Learning Objectives: 1) to describe the unique features of mammalian thermoregulation, 2) to emphasize the importance of behavior and passive heat transfer as thermoregulatory mechanisms, and 3) to propose a noninvasive alternative to general surface temperature manipulation as a means for effective treatment of heat- and cold-stressed individuals.

Abstract

The thermoregulatory system of mammalians is such that minimal energy expenditure is required to maintain a relatively stable internal thermal condition, despite large variations in environmental conditions and internal heat generation. Primary heat exchange occurs through specialized heat exchange-vascular structures that underlie the noninsulated body surfaces. In humans, these heat exchange-vascular structures are found exclusively underlying the palms of the hands, soles of the feet, the ears, and the hairless skin surfaces of the face. The treatment of hypo- and hyperthermia requires effective delivery of a thermal load to the body core. Heat may be delivered directly to the thermal core in a noninvasive manner via the heat exchange-vascular structures. To effectively utilize these structures, it is necessary to control blood flow through them. Future studies will focus on utilization of a combined application of subatmospheric pressure and an appropriate thermal load directly to the heat exchange surfaces of the hands and feet to treat heat- and cold-related maladies.

Mammalian temperature regulation has been the subject of scientific investigation since the advent of thermometers that could be inserted into an orifice of the body. Thousands of research articles, review articles, and book chapters have been written on the subject. A substantial portion of the *Handbook of Physiology: Section 4: Environmental Physiology*[1] deals with temperature regulation and is a comprehensive reference resource. Several more condensed,

Patents have been issued for the technology disclosed in this manuscript [D. Grahn and H.C. Heller (Inventors); Stanford University (Assignee)], and Stanford University has entered into licensing agreements with AVAcore technologies, Inc., and Dynatherm Medical, Inc., for the commercialization of the technology. Included in the license is a royalty agreement that grants Stanford University a percentage of the net sales of the technology, which will be shared by the University and the inventors. D. Grahn and H.C. Heller are founders of AVAcore Technologies but receive no ongoing compensation from the company.

Suspended animation for resuscitation from exsanguinating hemorrhage

Samuel A. Tisherman, MD, FACS, FCCM

Cardiopulmonary resuscitation with artificial respirations and external chest compressions have enabled initiation of life-saving interventions by lay persons and medical personnel, anywhere, anytime (1, 2). During normovolemic cardiac arrest, external chest compressions have a physiologic basis for efficacy. Open-chest cardiopulmonary resuscitation is physiologically superior (3, 4), although clinical studies have been inconclusive (5, 6). During exsanguination cardiac arrest, however, external chest compressions are not physiologically effective. Clinically, trauma victims who suffer cardiac arrest from exsanguination have almost no chance for intact survival, even after emergency department thoracotomy and open-chest cardiopulmonary resuscitation (7). Rapid attempts at fluid resuscitation and hemostasis lose the race against the tolerance limits for complete ischemia of 5 mins for the brain (8) and about 20 mins for the heart (8, 9).

The majority of soldiers killed in action in Vietnam without brain trauma had penetrating truncal injuries (10). They exsanguinated internally within a few minutes. Such casualties are still considered unresuscitable, although many have technically repairable injuries on autopsy. In 1984, Bellamy, a U.S. Army surgeon, and Safar met and pondered recent military casualty data and agreed that a novel approach was necessary (i.e., suspended animation). Suspended animation is defined as treatment to preserve the viability of the entire organism during ischemia, such as no flow (cardiac arrest) or low flow (shock). The goal is to induce suspended animation with hypothermia, drugs, and fluids. If instantaneous preservation of the viability of brain and organism could be achieved, one could buy time for transport and major hemostasis during clinical death, to be followed by restoration of blood volume and resuscitation, using cardiopulmonary bypass (CPB).

Suspended Animation Animal Outcome Studies

Since the late 1980s, researchers at the Safar Center for Resuscitation Research of the University of Pittsburgh have been engaged in systematic outcome studies in dogs for the development of suspended animation (11). In the initial series of experiments, Tisherman et al. (12–16) and Capone et al. (17) explored hypothermic preservation at tympanic membrane temperatures (Tty) of 15°C (deep hypothermia) or 5–7°C (profound hypothermia) after 30 mins of hemorrhagic shock at a mean arterial pressure 40 mm Hg. Suspended animation was induced by closed-chest CPB with hemodilution by crystalloids. After circulatory arrest of 60–120 mins, CPB was used for reperfusion and rewarming. Tty of 34°C was maintained for 12 hrs, controlled ventilation to 24 hrs, and intensive care to 72 hrs. End points included functional outcome in terms of overall performance categories (OPC 1 = normal, 2 = moderate disability, 3 = severe disability, 4 = coma, 5 = death) and neurologic deficit scores (0–10% = normal, 100% = brain death). Standardized necropsy included perfusion fixation of the brain and histopathologic damage scoring of 19 brain regions.

Profound cerebral hypothermia (Tty 5–7°C) induced at the beginning of exsanguination cardiac arrest improved neurologic outcome compared with that with deep hypothermia (15°C) (12, 13). The University of Wisconsin organ-preservation solution in the microcirculation during circulatory arrest did not add cerebral benefit over that achieved with standard plasma substitutes (14). These initial studies had been performed with standard CPB systems and systemic anticoagulation, which would be contraindicated after trauma. In a separate study, use of a heparin-bonded CPB circuit without systemic anticoagulation did not offset the beneficial effect of profound hypothermia (15). The optimal hematocrit during no flow under profound hypothermia is unclear (16).

The last study of this series was the most important (17). Sixty minutes of normothermic hemorrhagic shock was followed by rapid cooling using CPB and 60 mins of cardiac arrest at Tty of <10°C. Complete functional recovery was achieved, and, documented for the first time, the brains were histologically normal.

Clinically, CPB cannot be initiated within the critical 5 mins of recognizable cardiac arrest. A different approach is needed. Rapid placement of an aortic catheter could allow targeting of the brain and heart with a flush of cold fluid. A double-balloon catheter could allow differential flushing of the heart and brain while assisting with hemostasis.

Hypothermia Strategies. Subsequent studies have utilized a single-balloon catheter (Cardeon, Saratoga, CA) for flushing the aorta with isotonic saline, at a rate of 1–2 L/min, starting at 2 mins of no flow. Catheter design seemed to influence outcome; with the opening at the tip, the straight flush resulted in better outcome than that achieved using a catheter with the tip closed and the flush

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Key Words: hemorrhage; cardiac arrest; suspended animation; induced hypothermia; delayed resuscitation; dog

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through multiple lateral openings. This flush at 0–4°C could lower Tty by 3°C per minute. The outcome model used included rapid, controlled hemorrhage from aorta and vena cava over 5 mins to cardiac arrest (which was ensured by inducing ventricular fibrillation), and aortic cold saline flush started at 2 mins of arrest, with drainage via the vena cava catheter (Fig. 1). The period of circulatory arrest was varied from 15 to 120 mins (18–21) under preservative Tty levels decreasing from 34°C to 6–10°C. Reperfusion and rewarming were accomplished with closed-chest CPB, primed with Ringer's and dextra 40 in saline.

With cardiac arrest of 15 mins of no flow, saline flush volume of 25 mL/kg (a clinically feasible, portable volume) at 24°C (room temperature) achieved Tty of 36°C and, at 72 hrs, functional normality with histologic damage, whereas the same protocol with saline at 0–4°C achieved Tty of 34°C, and two of six brains were histologically normal (18). With cardiac arrest of 20 mins (19), aortic arch flush rapidly lowered Tty to 34°C and achieved survival to 72 hrs with functional normality and minimal histologic brain damage.

For cardiac arrest of 15 or 20 mins, the catheter balloon was inflated in the descending thoracic aorta for aortic arch perfusion. With longer arrest times, ischemia of the spinal cord, gut, and liver became apparent. Hind leg weakness was observed. The authors found that the most reliable flush method might be the simplest: flush via a large-bore cannula in the femoral or iliac artery to include the entire organism. For circulatory arrest periods of >30 mins, very large volumes of cold flush solution would be required. For example, for a 70-kg adult human, this would translate to 7 L of iced saline, which is feasible for ambulances or emergency departments but not for field medics. For cardiac arrest of 30 mins (20), the flush volume of saline at 0–4°C was increased to 100 mL/kg via the femoral artery to achieve a Tty of 28°C; this achieved functionally normal brains (in some dogs, even histologically normal brains).

Cooling to a Tty of 20°C, 15°C, or 10°C preserved the brain and organism to achieve intact survival (OPC 1) after 60, 90, and in some dogs, even 120 mins of no flow (21) (Fig. 2). All six dogs with cardiac arrest of 90 mins and a Tty of 10°C were functionally normal, with no or minimal histopathologic damage. One dog, after cardiac arrest of 90 mins, one after cardiac arrest of 60 mins, and one normal dog without cardiac arrest had normal cognitive function based on a battery of tests 3 months later. Of concern clinically, however, was that delaying the start of flush to 8 mins of arrest in the 30-min cardiac arrest model negated the preservation achieved with flush starting at cardiac arrest of 2 or 5 mins (22).

To achieve a Tty of 10°C in an adult human with the flush strategy above would require enormous amounts of ice-cold fluid, which would be impractical in the hospital and impossible prehospital. Another approach would be to start with a single, small flush to achieve mild cerebral hypothermia and then to recirculate diluted venous drainage blood, with or without an oxygenator, through a cooler-heat exchanger, to reduce Tty to profound hypothermia (11). Nozari et al. (unpublished observations) found that the recirculation strategy enabled intact survival with full neurologic recovery after 90 mins of cardiac arrest at least as reliably as the initially used one-way flush but with one tenth the volume.

Pharmacologic Strategies. Pharmacologic approaches with novel drugs and solutions would be advantageous for induction of suspended animation by synergizing with hypothermia and, perhaps, decreasing the volume of flush that is needed (23–27). Even if the aorta could be accessed and cold flush initiated within the first 5 mins of normothermic no flow and a drainage catheter inserted into the vena cava, the 10- to 20-L cold solution (0–4°C) estimated to be required for a 70-kg adult human to lower Tty to 10°C (and core temperature to about 20°C) would not be feasible in the field. Although difficult in the ambulance or hospital emergency department, such large amounts of solutions could be stored in a refrigerator.

The same Pittsburgh team conducted

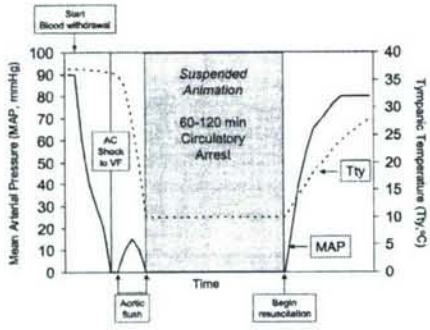

Figure 1. Model of exsanguination cardiac arrest, preservation via aortic flush, circulatory arrest for 15–120 mins, and resuscitation using cardiopulmonary bypass. *AC*, alternating current; *VF*, ventricular fibrillation.

	Overall Performance Category				
Arrest time	**1 Normal**	**2 Moderate Disability**	**3 Severe Disability**	**4 Coma**	**5 Dead**
15 min Tty 34°C	●●● ●●●	●			
20 min Tty 34°C	●●●●	●	●		
30 min Tty 28°C	●●●●	●●●●●*			
60 min Tty 10°C	●●●●				
90 min Tty 10°C	●●● ●●				
120 min Tty 10°C	●●	●	●	●	

Figure 2. Overall performance categories after exsanguination cardiac arrest of 15–120 mins with preservation via hypothermic aortic arch flush. *Tty*, tympanic membrane temperature. *Hind leg weakness.

the first systematic exploration of pharmacologic cerebral preservation potentials of 14 different drugs in 73 dogs (Fig. 3). The model used was 20 mins of exsanguination cardiac arrest with a potentially portable volume of flush solution (25 mL/kg) at ambient temperature, which achieved only mild cerebral hypothermia. In controls, saline flush started at 2 mins of cardiac arrest achieved survival with brain damage (19). In groups of three to six experiments per drug, various doses were flushed into the aortic arch via a balloon catheter, and in some experiments, additional intravenous medication was given during reperfusion with CPB. The drugs were selected and grouped according to six mechanistic strategies (26): 1) delaying energy failure, 2) protecting membrane integrity, 3) preventing structural degradation, 4) regulating protein synthesis, 5) preventing reoxygenation injury, and 6) preserving mitochondria. Selection of drugs and doses was influenced by published beneficial results (mostly in rodents) and guidance by expert consultants. Pharmacologic properties that would allow blood–brain barrier penetration were also considered. The goal was to identify a breakthrough effect (i.e., the majority of dogs in the miniseries to achieve OPC 1 at 72 hrs). None of the 14 drug treatments resulted in a breakthrough effect (23–25) (Fig. 3). Only an occasional dog achieved OPC 1 (but with some histologic damage) after thiopental plus phenytoin or glucose plus insulin. The antioxidant tempol, however, gave a suggestion of benefit (26). Tempol is available and inexpensive and penetrates the blood–brain barrier, but it is not approved by the U.S. Food and Drug Administration. All eight dogs that received 150–300 mg/kg tempol in the aortic arch flush at the start of cardiac arrest achieved OPC 1 or 2 (good outcome), whereas none of the eight control animals achieved good outcome (p = .03). Of concern, however, is that histologic damage was not significantly mitigated by tempol. Various explanations for this have been discussed (26). The only negative side effect of tempol, minimal transient methemoglobinemia, was clinically not significant.

	Overall Performance Category				
Drug	1 Normal	2 Moderate Disability	3 Severe Disability	4 Coma	5 Dead
Control	•	•••	••••••	••••	
Adenosine			••		
Thiopental	••		••	•••••	
Thiopental Phenytoin	•		••	••••	
Fructose Biphosphate			••	•••	
MK801			••	•••	
YM872			•	••	
Nimodipine			•	•	
Diltiazem			•	•	
Lidocaine			••	•	
Insulin Glucose	•		••	•	
W7			•	•	
Cycloheximide			•••		
Tempol	•••••	•••	•	•	
Cyclosporine A			•	•	

Figure 3. Overall performance categories after exsanguination cardiac arrest of 20 mins with preservation via aortic arch flush and novel pharmacologic potentials.

One may criticize this exploratory approach because it is not possible to rule out some benefit possibly revealed by larger sample sizes and randomized concurrent controls. The cost and time involvement needed to conduct such studies in large animals would be prohibitive.

Solutions. In the studies described above, isotonic saline solution was used for flush and dextran 40/Ringer's solution for reperfusion via CPB. Solutions designed specifically for profound hypothermia have been explored (27–30). Using the 30-min cardiac arrest model with Tty of 28°C (20), polynitroxylated albumin plus tempol (Synzyme, Irvine, CA) slightly improved neurologic deficit scores and histopathologic damage scores compared with saline, whereas 5% or 25% albumin did not (27). Using the 120-min cardiac arrest model with Tty of 10°C (21), Normosol (a pH-normalized Ringer's solution) was used for cold flush and "Unisol" (two solutions: an "intracellular fluid" with composition designed for stasis and an "extracellular fluid" designed for reperfusion), designed by Taylor et al. (29, 30) (Organ Recovery Systems, Charleston, SC), was used. With these "optimized" solutions, OPC 1 and only minimal to moderate histologic damage was achieved in five of six dogs. Additional studies to optimize the solutions are needed.

Trauma. Exsanguinating hemorrhage in trauma patients does not occur without significant tissue trauma. Nozari et al. (31) explored the above suspended animation approach with trauma added in the form of thoracotomy, laparotomy, and splenic transection. Splenectomy was performed during arrest. The coagulopathy due to hemodilution, hypothermia, and ischemia was greatly worsened by trauma, even with use of fresh donor blood during resuscitation. Nevertheless, exsanguination cardiac arrest of 60 mins plus severe trauma could be reversed to intact survival, but multiple organ failure occurred in several animals. The encouraging finding was that brain histopathology was normal. This suggests that, with prolonged intensive care and rehabilita-

tion (as could be utilized clinically), long-term intact survival would be expected.

Plasma exchange can decrease the microangiopathy seen in some patients with sepsis and multiple organ system dysfunction. Nozari et al. (unpublished observations), found that plasma exchange not only decreased the organ system dysfunction seen after trauma and suspended animation, but may also have improved neurologic outcomes.

Other Approaches. In addition to the Pittsburgh group, two other groups have explored the concept of suspended animation, although from somewhat different perspectives. Taylor et al. (29) and Bailes et al. (32) were interested in developing a method for protecting the brain during otherwise infeasible neurosurgical procedures. They showed that asanguinous low-flow perfusion of the organism with CPB of >3 hrs, under ultraprofound hypothermia (<5°C), could be survived with normal neurologic function. Specialized fluids were used during cooling, stasis, and resuscitation/rewarming. Long periods of total circulatory arrest were not explored, however. From a clinical perspective, in the exsanguinated trauma patient, intermittent low flow during suspended animation may be helpful for finding bleeding sites and, perhaps, improving preservation, although this remains to be explored.

Rhee et al. (33) have also explored suspended animation in a clinically relevant exsanguination model in pigs. Using readily available equipment, they induced profound hypothermia by aortic flush, both proximally and distally, via a thoracotomy and direct aortic cannulation. Repair of the aortotomy was accomplished during no flow. After total circulatory arrest of up to 40 mins, normal neurologic recovery could be achieved (33). The same group under Alam et al. (34) found normal cognitive function after exsanguinating hemorrhage from a vessel injury and prolonged asanguinous low flow (by CPB) at 10°C.

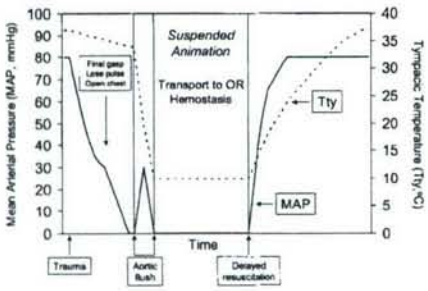

Figure 4. Possible clinical scenario for suspended animation in trauma victims with exsanguination cardiac arrest. As the patient becomes profoundly hypotensive, a last gasp and loss of pulse would be indications for rapid thoracotomy. If cardiac arrest is not rapidly reversed, the aorta can be cannulated and suspended animation can be induced via hypothermic flush to buy time for transportation to the operating room for control of major bleeding and delayed resuscitation using cardiopulmonary bypass. *OR*, operating room.

Cryobiology. Attempts at further extending the so far maximal duration of reversible cardiac arrest of 90–120 mins with hypothermia alone would take suspended animation research into cryobiology. Could one further extend the preservation time by going below 5°C? Profound hypothermia (5–15°C) has been shown in itself not to damage brain tissue (34, 35), but going below 5°C can cause denaturation of proteins and permanent cell damage, irrespective of the damage caused by ischemic anoxia (36). Ultraprofound cerebral hypothermia (<5°C) with special acellular synthetic solutions as blood substitutes, however, has been shown to preserve viability of rat hippocampus (36) and to achieve good outcome in dogs with low-flow CPB (32).

Future Directions

Potential Clinical Trials of Suspended Animation. For traumatic exsanguination cardiac arrest, clinical feasibility trials for the initiation of suspended animation are indicated, at least in emergency departments of major trauma centers (Fig. 4). Later, when appropriate devices become available for initiation of suspended animation outside the hospital, such feasibility trials could become part of emergency medical services research.

During severe hemorrhage without anesthesia, patients become unconscious when mean perfusion pressure decreases to <40 mm Hg, which is also about the point at which pulses are not palpable in large arteries. When apnea then ensues and pulsations are no longer palpable, one can assume cardiac arrest. Frequently, if the patient has had signs of life not long before this, emergency department thoracotomy is performed, particularly for victims of penetrating trauma. If a pulse cannot be rapidly restored, this could be a signal for accessing the aorta and administering the cold flush (i.e., inducing suspended animation). Drainage could be achieved rapidly by opening the right atrial appendage. The other potential approach for access would be cannulation of the femoral artery and vein via cutdown.

Given that the mortality rate for trauma patients who become pulseless from exsanguination and undergo emergency department thoracotomy is near 100% (7), clinical trials cannot be randomized. A reasonable approach would be to induce suspended animation after a brief period of unsuccessful resuscitation attempts, including thoracotomy and open-chest cardiopulmonary resuscitation. As clinical studies begin and experience grows, there are important questions that should be addressed. Who may benefit from expensive and labor-intensive suspended animation? What logistic problems need to be overcome to initiate suspended animation?

Device Development. To take suspended animation outside the hospital, devices for implementation will need to be developed. These devices should include a "smart catheter" to facilitate rapid percutaneous access to the aorta and vena cava, without thoracotomy and a miniaturized cooling-pumping device. Ideally, for portability in the field, the maximally miniaturized cooling source with pump could be developed for dual use: 1) for venovenous extracorporeal cooling for rapid induction of mild systemic or cerebral hypothermia in conditions with circulation (after normovolemic cardiac arrest, hemorrhagic shock, traumatic brain injury, stroke) and 2) for profound hypothermic aortic flush in conditions without circulation (i.e., suspended animation for cardiac arrest).

Other Applications. The main goal of suspended animation development has been to save some of the presently unresuscitable victims of traumatic cardiac arrest. It is worth keeping in mind that the suspended animation approach could also be useful when surgeons and anesthesiologists are unexpectedly losing ground with unmanageable hemorrhage during various surgical operations and for performing otherwise infeasible cardiovascular or neurosurgical procedures.

Summary

In dogs, isotonic saline at 0–4°C, flushed into the aorta at a rate of 1–2 L/min, with drainage of the vena cava, can achieve deep to profound hypothermia of vital organs at a cooling rate of up to 3°C per minute. This achieves preservation of viability of the organism during predictable durations of no flow: cardiac

arrest of 15–20 mins at Tty of 30–35°C, cardiac arrest of 30 mins at Tty of 25°C, cardiac arrest of 60 mins at Tty of 15°C, and cardiac arrest of 90 mins at Tty of 10°C. So far, pharmacologic approaches have not resulted in any breakthrough effect on outcome above that achieved with hypothermia, except perhaps the antioxidant tempol. Additional studies of novel drugs and, perhaps, combination therapies remain warranted. The optimal fluids to have in the circulation during circulatory arrest and reperfusions need to be determined. As laboratory studies to optimize suspended animation proceed, clinical trials should be initiated. In addition, devices should be developed to facilitate induction of suspended animation, eventually in the field.

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Smart aortic arch catheter: Moving suspended animation from the laboratory to the field

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The objective of the ongoing smart aortic arch catheter research and development program is to engineer "smart" catheter systems for enabling rapid vascular access and catheter placement, primarily within the aorta, for emergency hypothermia and suspended animation induction (1–6). The catheter systems are being designed and engineered to emphasize easy and rapid vascular access and catheter placement, in a compact and portable system, for use by civilian paramedics, military medics, or other trained first responders. The rapid vessel access devices will ultimately provide the necessary means for inducing suspended animation or preservative-resuscitative hypothermia, initially for use in hospital emergency rooms, then mobile intensive care unit ambulances or helicopters, and eventually for paramedics at the point of injury and in the field for combat medics.

The catheters will have the capability of delivering a large volume of cold (~2°C) saline flush into the aorta within several minutes. Immediate and targeted emergency hypothermia interventions may be able to isolate vital organs such as the heart, brain, spinal cord, and associated vasculatures and to impose a state of clinical preservation until transport can be provided to a facility for acute surgical care and delayed resuscitation. The smart catheter program encompasses stepwise design and development of smart catheter components for vascular imaging, trocar guidance and insertion, catheter placement, cold-flush connections, and monitoring of hypothermia by first responders in the field. Prototype catheter designs, aortic arch ultrasound imaging, three-dimensional position tracking of trocar and catheter tips, and system integration thus far have demonstrated the clear feasibility of rapidly accomplishing smart catheter placement for suspended animation induction. Specific catheter designs and guidance systems provide easy, rapid insertion and placement of catheters within the aorta and thereby facilitate the use of lifesaving emergency hypothermia for otherwise unresuscitable conditions. Initially, catheters are being designed and developed for 1) direct aortic insertion by the trauma surgeon in an emergency room via a thoracotomy site, 2) transthoracic aortic placement by a paramedic in the field using semiautomated ultrasound guidance and magnetic position tracking, and 3) aortic placement via femoral access by a paramedic in the field, initially by ultrasound guidance.

The successful design and development of a smart catheter and its guidance and placement system must provide easy-to-use, safe, and efficacious self-sealing, multiple-lumen, aortic balloon catheters, for both civilian and military trauma scenarios, with sufficient portability for field use at or near the point of injury. The aortic arch balloon catheter system will enable: 1) easy, semiautomated, foolproof insertion, sealing against the aortic wall via thoracotomy or transthoracic access, and guidance and confirmation of ascending, descending, or aortic arch placement; 2) rapid delivery of cold-flush solutions into the aorta from an external reservoir; 3) hypothermic preservation of the brain, heart, and spinal cord; 4) access for continued suspended animation and transition to cardiopulmonary bypass; and 5) access for optimal rewarming and transition to normothermic cardiac function.

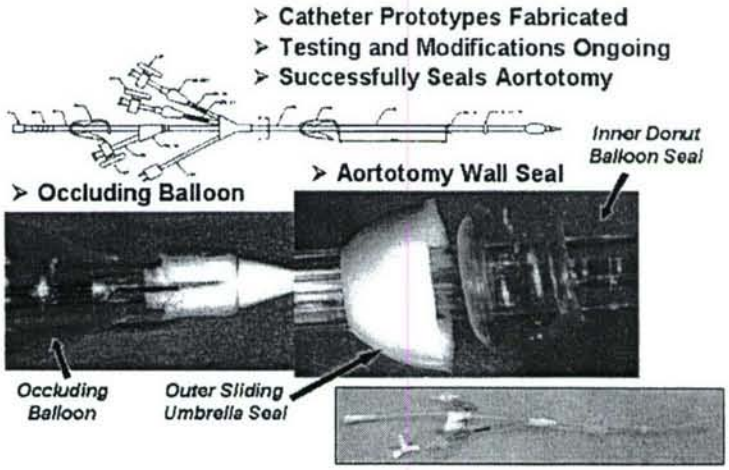

Figure 1. Thoracotomy catheter that has been designed and successfully tested in large animals. The catheter features include a mechanical seal for the aortotomy site consisting of an inner balloon seal and an outer sliding umbrella seal, which together compress the aortic wall to provide a tight seal. The aortic occluding balloon catheter provides aortic access for the delivery of cold-flush solutions.

From Alion Science and Technology, McLean, VA.

Key Words: suspended animation; hypothermia; delayed resuscitation; catheter; aorta; vessel access; portable ultrasound; magnetic position tracking

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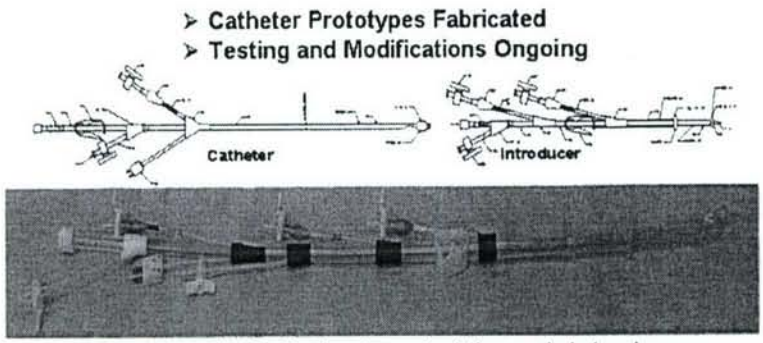

Figure 2. Similar to the thoracotomy catheter, the transthoracic catheter features include an introducer with a mechanical seal for the aortotomy site consisting of an inner balloon seal and an outer sliding umbrella balloon seal, which together compress the aortic wall to provide a tight seal. The transthoracic aortic occluding balloon catheter is then inserted through the introducer, as shown, to provide aortic access for cold-flush solutions.

Designs and Results

Catheters. Currently, the insertion of a catheter through the femoral artery into the aorta or directly into the aorta after left thoracotomy may be very quickly achieved. At trauma centers, surgeons are able to perform open chest heart massage in ≤1 min after confirming cardiac arrest and other options are exhausted. Similarly for closed chest scenarios, surgeons are able to cannulate the femoral vessels in humans within 3–4 mins during normovolemic cardiac arrest, while standard cardiopulmonary resuscitation is ongoing, well within the 4–5 mins before serious cerebral ischemic consequences. Ultimately, for exsanguinous no-flow, a direct femoral cutdown, left thoracotomy, or preferably, as proposed in this article, a smart catheter inserted transcutaneously is feasible and needed that quickly facilitates brain and heart cold flush. Rapid and easy vascular or aortic access is critical for the induction of emergency hypothermia and suspended animation at the point of injury to provide a brain and heart cold flush followed by continued fluid cooling. Even for experienced emergency room staff, identification and dissection of peripheral femoral vessels for insertion of arterial and venous catheters may take at least 15 mins in a pulseless patient, or even in a patient with low blood pressure. Typically, this emergency room intervention may be necessary to save the life of a victim using cardiopulmonary bypass for cardiopulmonary-cerebral resuscitation. The smart aortic arch catheter system is being designed for a fast, easy, and safe method to cannulate the aorta for targeted organ cooling. Catheter design and development has been ongoing and will continue by using approved materials for large-diameter balloon catheter and cannula designs. Both single and coaxial catheter designs have been explored. Simulation models have been constructed to produce breadboard configurations of the catheter and guidance systems working within closed-loop models of the aorta and phantoms for initial testing. Catheters and introducers have been fabricated with the assistance of Catheters and Disposables Technology (Minneapolis, MN).

For immediate interventional access, the smart catheter has been designed so that rapid access through the chest wall, from a parasternal approach, may be accomplished with subsequent direct insertion into the aortic arch. On insertion through the aortic wall, the catheter design includes the ability to provide a tight-sealing mechanism at the point of entry through the aortic wall to prevent fluid leaking from the aorta. Balloon-cuff concepts have been conceptualized and designed that may be adapted for this aortic catheter. An aortic arch catheter has been designed so that safe, easy, and rapid access to the aorta may be achieved through the chest wall from a transthoracic, percutaneous, or thoracotomy approach. Prototype, donut-shaped, balloon-cuff concepts have been designed and are used for this aortic catheter as one potential approach (Figs. 1 and 2). These designs maintain tight, leak-proof pressure on each side of the aortic wall. Ultimately, after delayed resuscitation, the point of aortic access would have to be closed surgically. Alternatively, access could be via the femoral artery, with a long catheter being extended to the appropriate position within the thoracic aorta or arch. The benefits of this approach include less potential damage to the aorta and the ability to have a lower placement of the catheter for increased cooling to the lower portions of the spinal cord and abdominal organs in the event of prolonged suspended animation, assuming the availability of adequate volumes of cold fluids.

Guidance and Placement System. For placement of the transthoracic introducer and catheter, portable ultrasound devices have demonstrated the ability to image the ascending aorta, the aortic arch, and the proximal descending aorta. Key images depend on suprasternal notch ultrasound probe placement. The ability to couple the image with access guidance and positioning of an introducer and catheter against the aortic wall was also demonstrated to be feasible using a bench-top prototype and ultrasound phantoms. Studies of the catheter placement challenge revealed the requirement for a location and placement capability based on ultrasound imaging integrated with three-dimensional position tracking. The initial details for integration of real-time ultrasound aortic arch images together with the trocar/catheter tip three-dimensional position have been developed. A software approach to provide this capability has been developed using ultrasound image and position data integration technology available through Cedara Software Corporation (Mississauga, Canada).

The smart catheter guidance, placement, and positioning system has been designed at this point to utilize three-dimensional ultrasound technology based on Cedara Software Corporation's Volume Explorer Framework technology. Position tracking has been successfully demonstrated using Ascension Technology's (Burlington, VT) miniBird magnetic tracking system. Although the smart catheter ultrasound system was initially configured to work with the Terason 2000 laptop-based portable ultrasound system (Terason, Burlington, MA), the current placement, positioning, and tracking system may be integrated with other portable or stationary ultrasound devices. The working prototype system is shown in Figure 3.

At this point in development, the demonstration prototype for the integrated

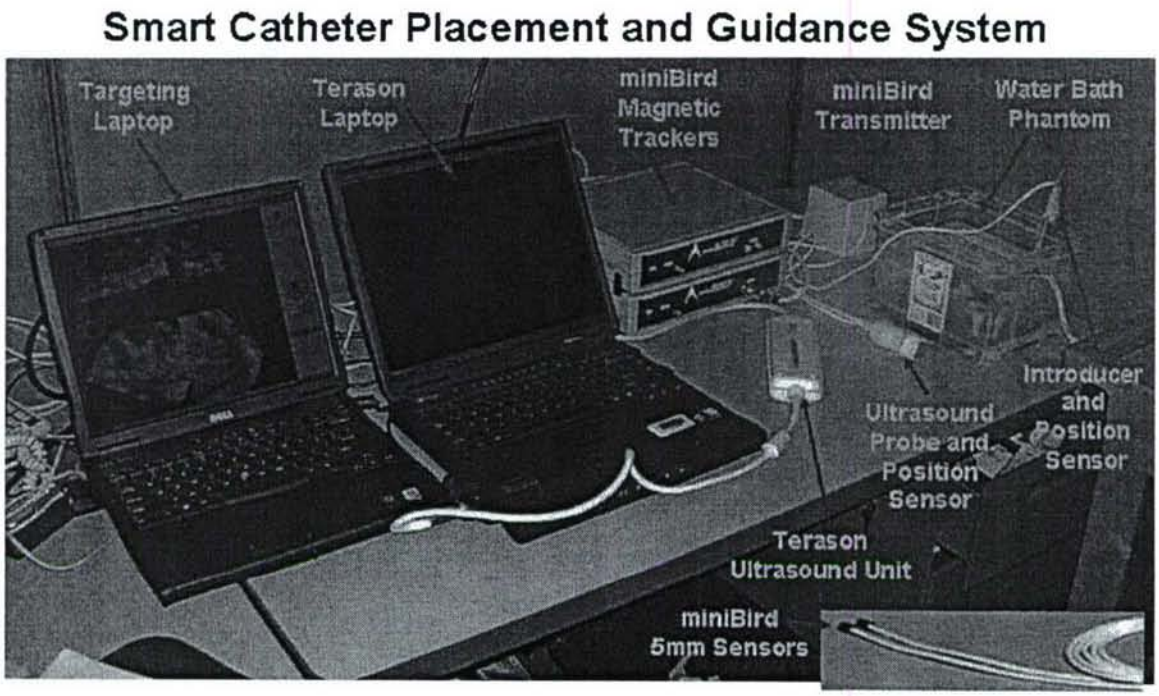

Figure 3. Current smart catheter guidance and placement demonstration prototype system, including the Terason 2000 portable ultrasound unit and ultrasound probe; the Ascension miniBird magnetic trackers, transmitter, and 5-mm position sensors; and ultrasound laptop and targeting laptop. Ultimately, all software and necessary interfaces will be integrated onto a single laptop or LCD for display.

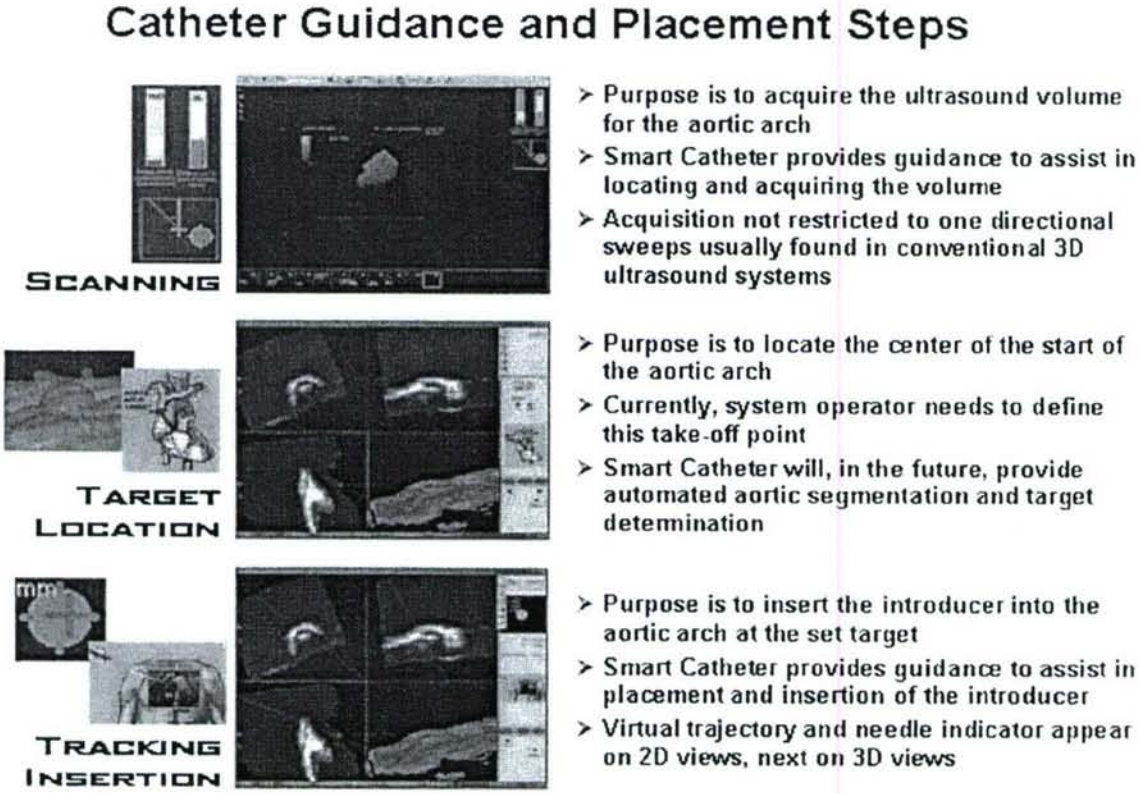

Figure 4. Three primary steps in the ultrasound-based guidance and placement system for the smart catheter into the aorta via a transthoracic approach include: scanning, target location, and tracking insertion. Ultimately, scanning will be continuous and in real time, target location will be fully automated based on aortic arch segmentation as is currently performed, and target insertion will be tracked in real time using a three-dimensional (*3D*) view and virtual trajectory for the introducer/catheter. *2D*, two dimensional.

Moving suspended animation from the laboratory to the field is now fully feasible and achievable in the near future.

guidance and positioning system includes: 1) the Terason laptop ultrasound system and ultrasound probe; 2) the Ascension miniBird magnetic tracker, transmitter, and 5-mm position sensors; 3) Cedara smart catheter–specific software; and 4) smart catheter introducer and catheter. The smart catheter software system divides the catheter placement and positioning procedures into three phases, including acquisition, targeting, and insertion. These functions are detailed in Figure 4, including computer interfaces displayed during the procedures. The design includes automatic target determination of the aortic arch point for catheter insertion. The system currently provides two user interfaces, one relatively complex interface displayed on the laptop and a second simplified interface displayed on a small LCD. Ultimately, when adequate resolution is available, a heads-up display will be employed to provide the user with catheter placement and positioning information.

The current system prototype seeks to incorporate a semiautomated to fully automated aortic/vascular target identification capability with image visualization enhancements. This is being accomplished through automated segmentation of the target of aortic ultrasound image followed by automated location of the catheter insertion target point on the wall of the ascending aorta. Ongoing work will also include the display of the introducer's trajectory in a three-dimensional view. Ultimately, for the transthoracic approach, a smart catheter "bib" concept, as shown in Figure 5, has been designed for stepwise development and will be prototyped. This smart catheter system bib will be placed on the chest and positioned to specific anatomic landmarks to aid in the positioning of the ultrasound probe, the placement of the magnetic positioning reference point, and the entry point for the catheter introducer.

Conclusions

The smart aortic arch catheter project goal is to meet the development challenge for field induction of suspended animation. Catheter seals have been successfully developed and tested, and the feasibility of an ultrasound based guidance, placement, and tracking system for the smart catheter has been demonstrated using the Terason laptop ultrasound system integrated with Ascension's miniBird magnetic position trackers and Cedara's three-dimensional ultrasound imaging and navigation software specifically adapted for the smart catheter system. Based on these initial design developments and prototype demonstrations, moving suspended animation from the laboratory to the field is now fully feasible and achievable in the near future.

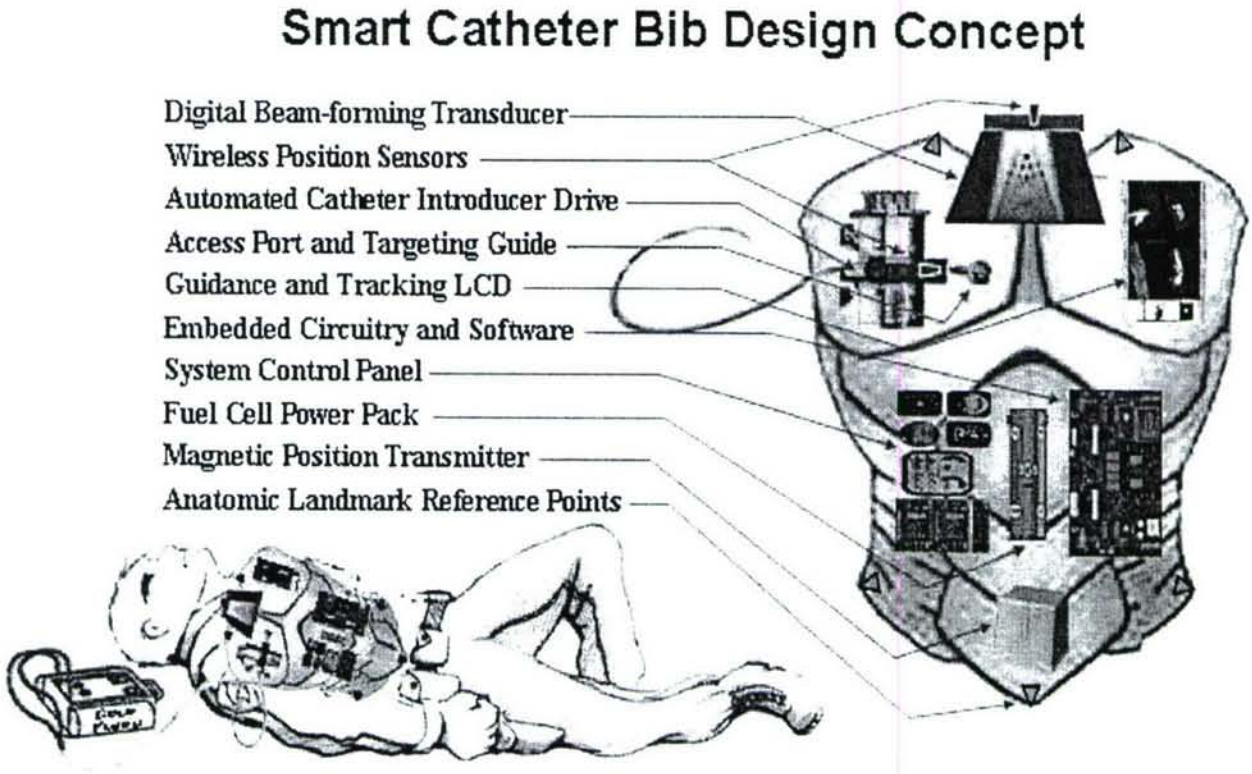

Figure 5. Smart catheter "bib" design concept that has been developed and will be prototyped in a stepwise fashion as key technologies become available. Initially, the bib will include only the ultrasound transducer, access port and targeting guide, guidance and tracking LCD, magnetic position transmitter, and anatomic landmark reference points.

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Novel Potentials for Emergency Hypothermia: Suspended Animation with Delayed Resuscitation from Exsanguination Cardiac Arrest

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*Dr. Peter Safar died on August 3, 2003. This chapter is written in his honor.

Abstract

Most combat fatalities result from rapid exsanguination in the field and resuscitation via fluid administration is generally unsuccessful. In a series of experiments over the past five years, we have developed a novel approach targeting the use of rapid induction of profound hypothermia by aortic flush to produce a state of suspended animation for delayed resuscitation after experimental exsanguination cardiac arrest in dogs. In over 200 experiments in dogs, exsanguination cardiac arrest was induced by rapid hemorrhage over ~5 minutes. After the 5-minute hemorrhage and an additional 2 minutes of cardiac arrest, hypothermia (8-34°C, tympanic temperature) was induced by aortic or femoral flush of ice-cold saline via a balloon catheter. Cardiac arrest was then continued for durations ranging between 15 minutes and 120 minutes. The specific duration and temperature selected depended on the goals of the specific study. Delayed resuscitation after the predefined suspended animation interval was achieved using cardiopulmonary bypass (for 1-2 hours), mild hypothermia (34°C to 12 hrs) and 72-90 hrs of continuous intensive care. In some studies, the insult also included laparotomy, splenectomy, and thoracotomy–to simulate trauma. In other studies, pharmacological agents were combined with hypothermia to test for therapeutic synergy. Final neurologic outcome was assessed at 72-96 hrs by overall performance category and neurological deficit scores. Brain histopathology was also evaluated. Normal neurologic outcome with minimal histopathologic damage was routinely achieved after a cardiac arrest of 90 minutes using this suspended animation approach. In some dogs, good neurologic outcome was achieved even after a cardiac arrest of 120 minutes. A delay of 5-8 minutes in the induction of suspended animation attenuated its preservative effect. Of 14 drugs

tested, only the antioxidant tempol produced a synergistic effect with hypothermia. The addition of trauma worsened organ function without affecting brain histopathology. Suspended animation with delayed resuscitation represents a revolutionary approach to resuscitation of the trauma victim with otherwise lethal exsanguination cardiac arrest. Our studies suggest additional benefit from the combination of antioxidants with hypothermia and challenge the previously posed limits of hypothermic protection and preservation of the brain. In ongoing studies we are testing suspended animation after prolonged shock, evaluating the mechanisms of hypothermic protection using proteomics, and probing beyond the 2 hr theoretical limit for cardiac arrest duration with intact survival.

Introduction

Most deaths in combat occur in the field from rapid exanguination, not from the development of post-resuscitation disorders such as multiple organ failure or sepsis. Colonel Ronald Bellamy (1) in his treatise on the cause of death in conventional land warfare discussed the fact that about 44% of combat casualty deaths in the Vietnam conflict resulted from wounds that produced exsanguination (often internal) in the field. In this subgroup of battlefield victims, many reached a military medic within minutes and a substantial number of the casualties killed in action had potentially reparable injuries. It is also well known that administration of large volumes of intravenous fluids in the field or on transport can be futile in this setting of rapid exsanguination, and results in massive hemodilution, and failure to prevent the development of exsanguination cardiopulmonary arrest (2). These facts suggested the need for a novel approach to the resuscitation. In 1984, Dr. Peter Safar and Colonel Bellamy subsequently pioneered the concept of an alternative approach that might salvage some of these combat victims, namely, induction of a state of "suspended animation" in these victims at the time of arrest, to be followed (after transport to a field hospital) by surgical repair and delayed resuscitation using cardiopulmonary bypass (3).

Studies on this project have been underway in the Safar Center for Resuscitation Research at the University of Pittsburgh School of Medicine since 1988. Despite the seemingly daunting nature of this approach—i.e., how might one consider successfully inducing a state of suspended animation, to preserve the brain, heart, and other vital organs for a period of time and successfully follow this with delayed resuscitation, Safar and colleagues –in a series of studies using a canine model of exsanguination

cardiopulmonary arrest—have outlined a putative approach to this otherwise lethal condition. This approach, which has been described in a series of reports (4-11), involves the rapid induction of profound hypothermia by aortic flush with ice-cold saline.

Exsanguination cardiac arrest model

In these studies, a canine model of exsanguinataion cardiopulmonary arrest has been used. This model has been described in detail in multiple reports—and has included various minor modifications depending on the specific goals of the study (4-9). Briefly, in studies approved by the Institutional Animal Care and Use Committee of the University of Pittsburgh School of Medicine and the United States Department of Defense, a protocolized and controlled rapid exsanguination was induced in halothane-anesthetized male dogs (custom bred hunting dogs, 20-25 kg body weight) using both femoral artery and right atrial catheters. Mean arterial blood pressure was 20 mmHg at 4 minutes. To ensure zero blood flow after 5 minutes of hemorrhage, ventricular fibrillation was induced with a transthoracic shock (5).

Aortic flush to induce suspended animation

In the initial studies of the induction of suspended animation for delayed resuscitation, after completion of the 5-minute exsanguination period described above, dogs were allowed to remain in a state of cardiopulmonary arrest for 2 additional minutes—without any intervention. After this 7-minute insult, the aortic arch was flushed (through a balloon catheter) with ice-cold (~4°C) saline (or room temperature [~22°C] saline in control groups) to rapidly induce various degrees of hypothermia

ranging from mild (34°C) to profound (10°C). Temperature was assessed using probes in multiple locations, but tympanic temperature was defined as the target, since this location is known to represent the best non-invasive means of approximating brain temperature (13). The target tympanic temperature was varied, as described above, depending on the goals of the study. Obviously, the flush volume and rate determined the time necessary to achieve the desired target temperature. In some studies, we fixed both the flush volume and flush temperature and allowed modest variation between animals in the level of hypothermia that was achieved. The time to target temperature varied—depending on the depth of hypothermia that was desired. In the studies where only mild or moderate (28-32°C) hypothermia was the goal, target temperature was often reached in less than 5 minutes. Even with prolonged suspended animation at profound hypothermia, the time to target temperature was generally less than 15-20 minutes. The duration of suspended animation was also varied in different experiments, again depending on the goal of the specific study. In early reports of the use of this approach, the duration of suspended animation was chosen to be either 20 minutes or 30 minutes (4,5), while in more recent studies, we have been able to successfully extend the duration of "suspended animation" to as long as 120 minutes (9).

Delayed resuscitation

After the desired interval of suspended animation, resuscitation was initiated by starting closed-chest cardiopulmonary bypass and the shed blood was re-infused. Mild hypertension was used during early reperfusion to promote cerebral blood flow (14) and re-warming was also achieved on bypass—but only to the level of mild hypothermia

(34°C) that was maintained for the initial 12 hours. After the initial 12 hours, normothermia was maintained until 72 hours. Cardiopulmonary bypass was gradually weaned during the initial 2 hours of resuscitation. During the initial recovery period, dogs received protocolized and titrated care that mirrored contemporary clinical intensive care and included continuous monitoring, mechanical ventilation, pressor and inotropic support with norepinephrine as needed, intravenous fluids, and sedation. Dogs were weaned from mechanical ventilation and extubated, generally on day 2 or 3. Monitoring and care was then continued in a step-down unit until sacrifice by transcardiac perfusion with paraformaldehyde at 72 hours. Details of the intensive care protocol are provided in a number of prior reports on this model (4-9).

Outcome assessment

Functional outcome assessment included overall performance category (OPC, 1 = normal, 5 = brain death) and neurologic deficit scores (NDS, 0-10% = normal, 100% = brain death). Daily, best, and final OPC were assessed. After paraformaldehyde perfusion, coronal brain sections were examined by a pathologist blinded to treatment group assignment. Nineteen brain regions were scored for damage to generate a histologic damage score (HDS, 0 = no damage, > 100 = severe damage), as previously described by our group (5,9).

Individual studies assessing the ability of suspended animation to preserve the organism after exsanguination cardiac arrest

Suspended animation of 20-30 minutes: Comparison of ice-cold vs room temperature flush

In the initial studies with this model we tested the effect of aortic flush of 500 mL of either room temperature (control) or iced (~4°C) saline (4). A tympanic temperature of ~36°C vs ~34°C was achieved with this modest aortic flush volume. Despite the use of only mild hypothermia, suspended animation induced with ice-cold flush significantly improved outcome as defined by each of the parameters defined above. For example, median OPC was 4 in the control group vs 1 in the ice-cold saline group ($p < 0.05$). Mean (±SEM) HDS were 109±39 vs 30±24 in the control vs ice-cold saline groups, respectively ($p < 0.05$). Using an identical protocol, an ice-cold saline flush volume of 100 mL/kg facilitated intact recovery from suspended animation of a 30-minute duration (5).

Pharamacologic agents to augment ice-saline in the induction of suspended animation

To determine if drugs could enhance the beneficial effects of hypothermia in this suspended animation paradigm; and thereby reduce the volume of flush required, we tested the effect of 14 different mechanism-based pharmacologic strategies in the aforementioned 20 min model of suspended animation. These therapies included agents targeting energy failure (thiopental or fructose bisphosphate), excitotoxicity (MK 801,YM 872, or Phenytoin), calcium accumulation (Nimodipine, Diltiazem, or W7), oxidative stress (Tempol), apoptosis (cyclohesimide), or mitochondrial permeability transition (cyclosporin), along with several other traditional agents (lidocaine, and

glucose/insulin). Overall, the effects of drugs were modest compared to the powerful effect of profound hypothermia in our paradigm (see below). Some of the results of this work have already been published (6-8). One drug, tempol, however, did significantly enhance the effect of hypothermia in this paradigm (8). Tempol is a cell permeable, stable, nitroxide antioxidant. It has direct radical scavenging effects, and also acts both as a superoxide dismutase mimetic and oxidizes Fe++ to prempt the Fenton reaction (8). Tempol given at a dose of 150 mg/kg or 300 mg/kg was detected in brain at 72 hours after administration by electron paramagnetic spin resonance and treatment improved functional outcome (OPC and NDS, $p < 0.05$ for tempol vs vehicle groups), but did not reduce histopathological damage (HDS). Although overall, our studies with pharmacologic approaches to augment hypothermia were generally disappointing, we recognize that it was not possible to carry out detailed studies of brain pharmacokinetics and pharmacodynamics of these agents in our dog suspended animation model. It was also not possible, given the limited tools available to study molecular mechanisms in dogs, for example, to demonstrate effects of therapies on key proteins in the apoptosis cascade, or mitochondrial injury. In addition, we did not study the effect of drugs in combination with profound or ultraprofound hypothermia. Pharmacologic adjuncts and optimal cooling solutions may differ for different target temperatures. Thus, these studies with pharmacologic agents represent an initial screening approach to combination therapy (hypothermia plus drugs) in our model. Our work suggests that therapies targeting oxidative stress represent an important area for additional exploration.

Suspended animation of 60-120 minutes

If suspended animation was induced in the field in the setting of exsanguination cardiac arrest, to be able to allow helicopter transport of an arrest victim to a field hospital (where both emergency surgery and cardiopulmonary bypass for delayed resuscitation would be available), a longer duration of suspended animation (than 20-30 minutes) was felt to be essential. In an initial investigation targeting the goal of extending the duration of suspended animation after exsanguination cardiac arrest that could achieve intact neurologic outcome, we tested the effect of cooling to profound or deep levels (10-15°C) of hypothermia using larger aortic flush volumes (9). This approach (use of profound or deep hypothermia) was taken because of the failure of drugs to dramatically improve the efficacy of moderate hypothermia, as described above. First, we compared large volume aortic flush to induce either deep (20°C or 15°C) or profound (10°C) hypothermia in our suspended animation model studying a 60-minute duration. A temperature of 20°C failed to achieve normal outcome, while, either (15°C or 10°C) produced normal functional and histologic outcomes (OPC 1 and NDS 0-3%, HDS 0-20)(9). However, to achieve these temperatures (20°C, 15°C, or 10°C) required, on average, flush volumes of 160, 308, and 482 mL/kg, respectively. Normal outcome after 90 minutes of suspended animation in our exsanguination cardiac arrest model was achieved with a brain temperature of 10°C. This was produced with a flush volume of over 500 mL/kg. Using profound hypothermia (tympanic temperature of 10°C), and a flush volume of over 600 mL/kg, suspended animation of 120 minutes lead to normal functional and histologic outcome in some, but not all dogs (9). In these studies of the application of suspended animation for prolonged durations (≥90 minutes), it became necessary to optimize whole body cooling by initially cooling brain to target (tympanic)

temperature, followed by deflation of the aortic balloon, and withdrawal of the catheter to a position in the abdominal aortic and continued optimal cooling of the structures subserved by the distal aorta—particularly the spinal cord and gut (9). These remarkable studies demonstrate the potent preservative and resuscitative effects of profound hypothermia in the setting of exsanguination cardiopulmonary arrest.

Reducing the fluid volume required to induce suspended animation

For the field application of a therapy such as suspended animation, unlimited quantities of ice-cold saline would not be available. Thus, we sought to develop a strategy that would reduce the flush volume required. To this end, veno-arterial re-circulation of the cold flush was instituted (10). The venous drainage from the right atrium was re-circulated, cooled and re-infused into the aortic catheter. This approach dramatically reduced the flush volume necessary to achieve deep or profound hypothermia. In that study, we also determined that aortic flush could be successful whether it was initiated in the distal aorta (femoral catheter) or the aortic arch (10). Clinically, the re-circulation approach may be limited by disruption of major blood vessels.

Superimposing trauma onto the suspended animation paradigm

Recent work in our center has focused on maximizing the clinical relevance of this suspended animation approach, in an effort to refine and optimize this therapy for a potential clinical feasibility trial. Since exsanguination cardiopulmonary arrest is produced by initial injury (usually penetrating), tissue trauma comprised of a splenic

laceration, laparotomy, thoracotomy and (delayed) splenectomy was superimposed onto the suspended animation paradigm. To further maximize the clinical relevance of the experiments (mimicking field induction and prolonged transport), a 120-minute interval of suspended animation (at 10°C) was used. This combined insult, however, was associated with the development of multiple organ failure, coagulopathy and death or poor outcome (11). To treatt the coagulopathy and multiple organ failure that developed in this setting, we added plasma exchange therapy to the protocol (three single volume exchanges during the initial 24 hours of the resuscitation phase) to achieve survival with good neurologic outcome. Details of this recent work were recently presented at the 2004 Congress of the Society of Critical Care Medicine in the United States (12).

Future directions

Current studies in our laboratory are investigating the impact of superimposing a prolonged period of hemorrhagic shock into the model before the induction of suspended animation—asking the question "Is the patient who has been in shock deteriorates to pulselessness, is suspended animation still feasible?" This scenario mimics a soldier or civilian who develops hemorrhage-induced cardiopulmonary arrest over a more protracted period of time (1-2 hours rather than 5 minutes) (15). Additional laboratory work underway is also assessing the proteins damage during the period of prolonged hypothermia—using proteomics (16). We also plan to again address the issue of optimal drugs and fluids to develop a better suspended animation solution than normal saline. Finally, as discussed above, we hope to begin planning for the first application of suspended animation in the setting of civilian exsanguination cardiopulmonary arrest.

We are also working with industrial partners to aid in the development of "smart catheters" necessary for rapid cannulation of the aorta and devices needed for rapid cooling in patients (17).

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A Novel Approach to Resuscitation: Suspended Animation for Delayed Resuscitation

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Since its appearance in the utopian novel *Looking Backward* by the American author Edward Bellamy in 1888, "Suspended Animation" (SA) remained a hypothetical technical term in science fiction for nearly a century. In 1984, Dr. Peter Safar, the Father of Modern CPR, and Dr. Ronald Bellamy, a US Army surgeon, redefined SA and consolidated the concept with sound scientific rationales and potential practical values in medicine [1]. By the end of the last century, a series of experiments, mostly conducted in the Safar Center for Resuscitation Research at the University of Pittsburgh, showed that SA is feasible in a large animal model of cardiopulmonary arrest (CA), and that SA might ultimately be feasible in a clinical setting [1]. To appreciate the scientific concept of SA, a brief review of current resuscitation of traumatic CA is helpful.

The Challenge of Traumatic CA in Resuscitation

In the civilian setting, 50% of deaths due to trauma occur at the accident scene, with another 30% occurring within a few hours of injury [2]. In the military setting, a similar picture of rapid deterioration has been reported. For example, the majority of the US soldiers killed in action in Vietnam without brain trauma had penetrating truncal injuries and exsanguinated to CA within minutes [1]. These injuries were often surgically repairable. Unfortunately, for a large portion of the acute mortality from rapid exsanguination CA in both civilian and military settings, no effective methods have been established to reliably improve survival. The technical obstacles are formidable. Shortening rescue and transport time barely impact the irreversible deterioration of the brain that occurs 5 min after CA. The need for surgical repair in these patients is also obvious, but not feasible in the setting of exsanguination CA. Finally, conventional CPR is generally futile in these cases because of a volume-depleted and trauma-disrupted circulatory system. More aggressive treatments with thoracotomy and aortic cross clamping have also not improved the poor outcome in these patients [3]. The grim prognosis has led the National Association of EMS Physician Standard and Clinical Practice Committee and the American College of Surgeons Committee on Trauma to publish guidelines for termination of resuscitation in pre-hospital traumatic cardiopulmonary arrest. If patients with either blunt or penetrating trauma are found apneic, pulseless, and without pupillary reflexes,

or spontaneous movement, termination of resuscitation efforts is recommended [4]. In the military setting, resuscitation efforts are generally stopped if a patient fails to respond to 1 L of hetastarch [5].

Few studies have tried to address this problem. One group proposed the use of intra-aortic balloon catheter to stop bleeding beyond the descending aorta and infuse blood or other solutions into the aorta above the balloon to avoid ischemic injuries in the heart and the brain [6]. This approach is similar to thoracotomy and aortic cross-clamping, but less invasive. However, outcome studies of this technique are lacking. This chapter focuses on a unique resuscitation concept, which is called SA for delayed resuscitation that we have been developing over the past 20 years.

The Concept of SA

Facing the challenge of traumatic CA, Drs. Safar and Bellamy jointly created the concept, SA in 1984. Instead of futilely trying to restart the circulation in the setting of exsanguination CA, preservation was proposed as the initial intervention to buy time for transport and surgical repair. After the anatomic defect in the circulation is repaired, cardiopulmonary bypass (CPB) is initiated as part of a delayed resuscitation.

Development of SA Experimental Models

Different from profound hypothermic CA in neurological and cardiovascular surgery, a clinically-relevant SA model should have these two key features: 1) the pre-existence of insults at normal or near normal body temperatures, including trauma, hemorrhage, and CA, and 2) a trauma-disrupted circulatory system that makes fluid replacement and chest compression futile. Any technology that relies on intact circulatory system, such as CPB, cannot be relied upon for induction of hypothermia in some cases. Because of its complexities, a step-by-step approach had been taken in the establishment of SA models.

1980s: Feasibility of organism preservation after hemorrhagic shock (HS): In the late 1980s, Tisherman *et al* conducted a ground-breaking series of SA experiments in dogs. The pre-existing insults were 30 or 60 min of HS at a mean arterial pressure (MAP) 30 or 40 mmHg. Hypothermic preservation was induced by closed-chest CPB with hemodilution using crystalloids. At the end, delayed resuscitation was performed with CPB. Profound cerebral hypothermia (<10°C) induced at the beginning of a 60-120 min

exsanguination CA improved neurologic outcome, vs deep hypothermia (15°C) [7]. [8-10] This series established the premise for the SA concept by showing that the pre-existing HS did not obviate the potential efficacy of hypothermic preservation.

1999-2002 Clinically relevant SA model: without trauma: The second series, again carried out in dogs, took one step closer towards the ideal SA model in that 1) SA was initiated after CA, assuming that most victims would have CA when approached by paramedics, and that people would be more willing to initiate SA after rather than before CA; 2) 2-5 min of CA was allowed to elapse, assuming the time that was required for vascular cannulation; and 3) one-way flush was used, assuming the existence of the disrupted circulatory system that does not allow CPB to function properly. The solutions were administered into the aortic arch using a balloon catheter, and drained out from the right atrium via an external jugular catheter.

Typically, the SA model that was developed has 3 phases: 1) a hemorrhage and CA phase: 5 min of rapid exsanguination followed by 2-5 min CA; 2) a SA phase: up to 3 h of preservation; and 3) a delayed resuscitation phase: initiated with 2 h of CPB for re-warming and return of spontaneous circulation, followed by up to 96 h of intensive care. The final outcome is assessed at 72 or 96 h based on evaluation of Overall performance Category (OPC, 1-5) and Neurologic deficit score (NDS). A histological deficit score (HDS) was also developed which quantifies neuronal damage in 19 brain regions.

2002-2003: Clinically-relevant SA model: with trauma: The success of SA in non-trauma models prompted us to explore whether SA would work in the setting of experimental trauma with a superimposed exsanguination CA. Nozari *et al* added trauma in the form of a thoracotomy, laparotomy, and splenic transaction into the above SA model in dogs [11]. Splenectomy was performed during the arrest that followed. As expected, coagulopathy due to hemodilution, hypothermia, and ischemia were greatly worsened by trauma, even with use of fresh donor blood during resuscitation. Nevertheless, 60 min of CA plus severe trauma could be reversed to intact survival in about 50% of the dogs. In the rest, multiple organ failure (MOF) occurred, and evaluation of neurologic function was not possible. Histology revealed that there was almost no brain injury in any of the dogs. In subsequent studies, based on studies in clinical

sepsis and MOF showing efficacy of plasma exchange in decreasing the microangiopathy, Nozari *et al.* reported that plasma exchange not only decreased the MOF seen after trauma and SA, but also had improved neurologic outcomes after 2 h of exsanguination CA [12].

Therapeutic Windows for SA

Two studies had been conducted to explore how much the pre-existing duration of CA or HS limit the efficacy of SA. In a 30 min CA model, initiation of 2°C normal saline flush was delayed by 2, 5 or 8 min from the onset of CA. All dogs received 100ml/kg normal saline flushed into the thoracic aorta via a balloon catheter over 4 min. Delays in flush did not change the efficacy of brain cooling. When cooling was delayed by 2 or 5 min, all 12 dogs regained consciousness after resuscitation. In contrast, those with 8 min delay remained comatose and severely disabled [13]. This suggests that the time window for the onset of the flush is between 5 and 8 min for SA to be successful after CA.

All of the initial work with SA used a paradigm in which exsanguination CA was rapidly induced - over 5 min. This was done to model the rapid exsanguination that was observed in the battlefield in lethal combat casualty from gunshot wounds. However, not all exsanguination CA occurs rapidly. Thus, it was not clear if SA would be effective if induced in the setting of an exsanguination CA that had developed over a much longer period of HS such as 1-2 h or more. To answer this question, dogs were gradually but continuously hemorrhaged until CA, using a paradigm that resulted in CA at between 1.5 and 2.5 h after the onset of hemorrhage. Before CA, about 60 to 90% of the estimated total blood volume was removed [14]. Upon CA, the arterial blood gases revealed the following mean values for key physiological parameters including 1) pH ~7.0, 2) BE ~-15 mmol/L, 3) lactate ~15 mmol/L, and 4) K^+ ~ 7.0 mmol/L. After 2 min of CA, either conventional CPR or SA was initiated. Convention CPR included chest compression, pressure-controlled ventilation, and vigorous volume replacement. In contrast, SA was rapidly induced with flush of 20 L of ice-cold saline via a femoral artery, and maintained for a period of 60 min. We found that while all dogs treated with CPR died before 16 h, all but one dogs treated with SA survived to >72 h. Surprisingly, to produce intact neurological outcome in this paradigm, it was necessary to follow SA with a 36 h period of

mild hypothermia (34°C). Thus, prolonged HS prior to exsanguination CA should not preclude the possibility of survival with intact neurological outcome after SA treatment.

The Development of Hypothermic Approaches for SA

Hypothermia so far is the most reliable and potent approach for preservation during CA. Behringer *et al* conducted a series of experiments that systemically explored specific details of the optimal hypothermic approach [15][16-18]. As shown in Table 1, a linear relationship exists between brain temperature and effective preservation durations (r=0.97) in SA. To achieve 20-min preservation, cooling to brain temperature of 34°C is needed; for 30 min, 28°C is needed; for 60 min, 15°C appeared sufficient; while for 90 min, ≤10°C is needed. The efficacy of ice-cold saline for cooling, however, decreased dramatically as the brain temperature decreased. During a temperature reduction from 37 to 15°C, 1 ml/kg ice-cold saline reduced brain temperature by ~0.16°C; from 15 to 10°C, however, the same amount of cold fluid reduced brain temperature by only ~0.018°C/ml/kg—a reduction in cooling efficiency by nearly a factor of ten. When Tty was reduced to 7°C, the effective preservation time appeared to be 2-3 h, but the requirement of large amount of fluid to achieve this temperature in our paradigm limits its application on the field.

Alam *et al* [19] studied the impact of rate of cooling on outcome in a model of large blood vessel laceration in pigs. Thirty min after vascular injury, cooling with CPB was initiated to achieve a pharyngeal temperature of 10°C at an averaged speed of 0.5, 0.9, and 1.35°C/min. The CA lasted for 60 min, followed by re-warming and delayed resuscitation. Six weeks later, the survival rates were 37.5%, 62.5%, and 87.5% respectively, supporting the notion that the most rapid cooling rate is maximally efficacious. However, it is unclear if cooling at an even faster rate (>2°C/min) would further improve outcome. We found one-way flush with ice-cold saline was faster in cooling than CPB. At ~1.2 L/min with one-way flush, the cooling rate (to achieve a temperature of 10°C) was about 2.5°C/min [18]. Surprisingly, cooling with CPB (recirculation), although slower, appeared to yield better neurological outcome [20]. Although there were other factors that may be responsible for the differences in outcomes, some transplantation researchers

believed flush solutions that are administered at very low temperatures can be detrimental for organ preservation [21-22]. The optimal cooling rate for induction of hypothermic SA remains to be determined.

The rate of re-warming from profound hypothermic SA also influences outcome. Using CPB, Alam *et al* induced 10°C hypothermic CA in their pig vascular injury model [23]. When pigs were re-warmed at the maximal rate that was technically achievable (~ 0.52-0.8°C/min depending on core temperatures), the 6 week survival rate was only 30%, in contrast to 90% survival with somewhat slower re-warming at 0.5°C/min. Surprisingly, substantially slower re-warming at 0.25°C/min also produced poor outcome (6 week survival rate 50%). We have not set a target re-warming rate. Instead, the CPB water bath is set at a temperature that is 5°C higher than the core blood temperatures. In ~45-60 min the dogs were re-warmed to 34°C from 10°C. This approach results in an average rate of re-warming of ~0.4-0.53°C/min, which fortuitously has produced good outcome and appears to agree with the work discussed above [23].

Maintenance of mild hypothermia during the delayed resuscitation phase also appears to be a crucial adjunct for SA. Empirically, mild hypothermia at 34°C was kept for 12 h after CPB and satisfactory outcomes can be achieved with this approach for exsanguination CA that occurs rapidly. As discussed above, longer periods of mild hypothermia are needed when prolonged HS precedes exsanguination CA. In this setting, shorter (12 h) periods of mild hypothermia were insufficient and resulted in delayed neurological deterioration with seizures after re-warming. When hypothermia was extended from 12 to 36 h, there were no seizure and intact neurological outcome could be achieved even after an exsanguination CA that was preceded by over 2 h of HS and profound acidosis at the time of SA induction [14].

The Exploration of Pharmacological Approaches [Table 2]

The successful development of pharmacological adjuncts to SA could have dramatic benefits on the potential clinical application of this therapy. Drugs can be easily delivered into the circulation—in contrast to the large fluid volumes needed cool to levels of profound hypothermia (an aortic flush of ~500 mL/kg in dogs). If a pharmacological agent could completely supplant the need to cool, it would similarly eliminate the need to rapidly cannulate the aorta to administer a flush solution for SA induction.

To this end, we tested the effects of 14 different pharmacological approaches in our SA paradigm (Table 2). The model used was 20 min of exsanguination CA with a potentially portable volume of flush solution (25 ml/kg) at ambient temperature, which achieved only mild cerebral hypothermia. In controls, saline flush started at 2 min of CA produced survival, but with brain damage. In groups of 3 to 6 experiments per drug, various doses were flushed into the aortic arch via an intra-aortic balloon catheter, and in some experiments, additional therapy was given during reperfusion with CPB. The drugs tested were categorized into one (or more) of the following mechanistic strategies: 1) delaying energy failure, 2) protecting cell membrane integrity, 3) preventing structural degradation, 4) regulating protein synthesis, 5) preventing re-oxygenation injuries, and 6) preserving mitochondria. Remarkably, none of the 14 drugs yielded a breakthrough effect. The brain penetrating antioxidant tempol, however, appeared to produce some benefit [24]. Tempol is available and inexpensive and penetrates the blood–brain barrier, but it is not approved by the US Food and Drug Administration. All 8 dogs that received 150–300 mg/kg of tempol via the aortic arch flush, beginning at 2 min after CA, were normal or near normal, whereas none of the 8 control dogs achieved consciousness. However, histological damage was not mitigated by tempol [24].

The goal of this series was to screen for a breakthrough drug. Clearly, no single pharmacological agent was effective, and we concluded that the efficacy of drugs paled in comparison to hypothermia. However, synergistic effects of drugs with hypothermia are likely, and should be explored in future studies. In addition, we did not carry out studies examining brain pharmacodynamics—and it is thus unclear if each agent was able to penetrate the blood-brain barrier and target the specific mechanism and produce its desired biochemical or molecular effect. Further studies in this regard are needed.

The Exploration of Novel Preservation Solutions

In the history of organ preservation for transplantation, preservation solutions have played a key role. However, in our pilot experiments, various solutions seemed to offer only marginal effects. In a 30 min CA model, we found that neither 5-25% albumin nor Unisol (Organ Recovery Systems Inc., Pittsburgh, PA) improved outcome. In contrast, a combination of polynitroxylated albumin (Synzyme, Irving, CA) and

tempol significantly reduced NDS and HDS compared to Unisol [25]. In on study using a 120 min CA model, we evaluated a provocative approach put forth by Taylor et al [26] using a solution with an intracellular composition to fill the vasculature after the flush (Unisol-I [Intracellular]) and followed this with an extracellular based solution (Unisol-E [Extracellular]) to initiate delayed resuscitation. This approach achieved consciousness survival in 5 out of 6 dogs, while normal saline was less successful [15]. In a recirculation study of SA, that was originally designed to reduce fluid requirement, a better outcome with recirculation of diluted blood but without gas-exchange was found over the one-way flush [20]. This observation fuels the speculation that certain components in the blood may be beneficial. As there was not fresh oxygen supply, oxygen carrying capacity of hemoglobin did not produce the observed effects.

In the field of cardiac surgery, Aoki *et al* found that intermittent flush of University of Wisconsin solution via the carotid artery over 2 h of a profound hypothermic (15°C) CA in piglets improved recovery of cerebral blood flow and ATP during early reperfusion, compare to the saline flush group [27]. In an outcome study that followed, however, the piglets that received 50 ml/kg University of Wisconsin solution flushed via the carotid artery, exhibited similar OPC and NDS on day 5, but actually had worse histological deficit versus control that did no receive any flush during CA [28]. Robbins *et al* found that oxygenated "cerebroplegia" solution, which contained 2.5% glucose, 12.5% mannitol, 22mEq sodium bicarbonate, 25 mEq/L lidocaine, 0.5ug/L nitroglycerin, and 5 mg/L calcium chloride, flushed intermittently via the carotid artery during hypothermic CA substantially delayed brain energy depletion [29]. There was no outcome study published. More recently, Taylor et al [26] developed an asanguineous solution for whole-body perfusion during profound hypothermic CA. The choices of the components for the solution were literature-based, and satisfactory outcome after 3 h CA was achieved. However, it is not clear if the solution has any specific brain preservation effects, since the main problems seen in the controls but absent in the treatment group were cardiac or peripheral nerve injury and/or dysfunction [26].

Devices

Although we believe SA can be implemented in trauma centers using currently available surgical devices, to induce SA in the field requires the development of a number of related technologies. Most important is the need for the safe and timely cannulation of the aorta by paramedics-- who generally lack surgical expertise. For this, Yaffe *et al* have been developing a "smart catheter" --an ultrasound-guided approach with a self-sealing catheter that might allow paramedics to percutaneously insert an aortic catheter in the field [30]. Prototypes are also under development for portable cooling and pumping in the field.

Mechanistic Studies

Studies in large animals: The most vulnerable organ during prolonged CA, with or without hypothermia, is the brain. When the whole body was cooled to ~10°C for preservation of up to 3 h of CA, lethal extra-cerebral organ injuries were rare using our delayed resuscitation protocol. The mechanisms of brain injury in SA and reperfusion are likely multifactorial. Even when the brain temperature was reduced to 8°C, oxygen consumption remains at ~11% of baseline [31], and ATP and creatine phosphate in the brain was shown to be depleted in 60-90 min during deep hypothermia (12-15°C) [29]. Given that 2 min of normothermic CA precedes the induction of hypothermia in a SA model, energy depletion would occur sooner and may play a major role in the brain injury in our SA model. Release of excitatory amino acids and production of nitric oxide contribute to neuronal injury during profound hypothermic CA [32].

Proteomic approaches in SA: An initial study in our center focused on the degradation of brain proteins during a prolonged hypothermic or normothermic circulatory arrest. We noted that 30 min of complete ischemia at either 37 or 10°C results in minimal protein degradations as assessed with 1D and 2D gel electrophoresis [33]. Future studies will evaluate the effect of reperfusion on protein degradation. In 1960s, in seminal studies in the field of prolonged hypothermic preservation, White *et al* [34].preserved the dog brain at 2°C for hours to days. Remarkably, he used on isolated dog head preparation. After recirculation and re-warming, EEG signals, pupil light reflex and rhythmic gasping were seen in the heads that were preserved for 4 h. However, this electrophysiological activity eventually disappeared as reperfusion at 34°C

went beyond 6 h [34]. Incredibly, thus, in 1966, Whites' finding was consistent with the contemporary concept of "reperfusion injury" as a key mechanism of damage in brain ischemia.

A rat model of SA: We chose a dog model for these SA experiments to maximize clinical relevance. However, certain limitations are pertinent to that model. First, there are few molecular tools available for dogs. That limits the evaluation of impact of therapies on the cellular and molecular mechanisms of secondary injury. Understanding molecular mechanisms of ischemia-reperfusion injury, and the impact of SA on these cascades, would allow us to define specific targets for future interventions, and to assess markers of reversibility. Second, the cost and labor-intensiveness of the SA experiment in dogs poses an obstacle to rapid screening of drugs and preservation solutions that would seem promising.

Based on these limitations, we have recently launched a project to develop a rat model of SA to address these drawbacks. The cornerstone of the successful establishment of the model was development of a miniaturized CPB machine that would be effective in rats, because CPB is essential to delayed resuscitation after prolonged periods of SA.

In the past, there were many attempts to develop CPB for small animals[35];—the first one being used in cats as early as in 1937. Over the past sixty years, tremendous improvements have been achieved. Both pulsatile and non-pulsatile models of CPB have been described and tested on various large animals – dogs, cows, sheep, pigs and rabbits. In many studies, perfusion of isolated organs was evaluated, while only a few studies actually implemented CPB in small animals. Often, open-chest cannulation was used, which prevented long-term survival. A limited number of papers reported successful separation of the study animal from CPB. Recently, several centers reported successful establishment of CPB in rats[36]. Unfortunately, many of these reports are still published only in abstract form.

The absence of commercially available CPB machine for rodents remains another limiting factor. The static priming volume required in even the smallest pediatric oxygenators approximates 40 ml, which is nearly twice the total blood volume of a rat. Donor blood for the circuit prime is still necessary, even for the custom-made devices that are now available, with priming volumes less than 10 ml. Transfusion of whole

blood between rats does not appear to be an important problem with regard to blood compatibility; however, subtle transfusion-related injury may remain underappreciated.

Grocott and co-workers have reported that CPB *per se* causes neurologic and neurocognitive impairment in rats—even in the absence of CA[37,38]. CPB has also been shown to trigger mesenteric endothelial dysfunction[39], and acute lung injury[40] as part of the systemic inflammatory response syndrome. Mechanical ventilation itself leads to endothelial damage, especially superimposed upon ischemia-reperfusion injury[41]. These findings are in accordance with the well-recognized effects of CPB seen in humans. The leading cause of the neurologic injury associated with CPB use in man–microembolism–has not been studied in rats. Current research by investigators using these new CPB devices in rats is focused on the effect of CPB on the brain[42] and is targeting approaches to ameliorate the systemic inflammation[43-45].

As discussed, a proteomic approach was used to assess protein degradation after SA using decapitation to produce global ischemia. Those studies focused solely on the effect of intra-ischemic protein degradation during prolonged global cerebral ischemia with and without hypothermia. The model used precluded the possibility of reperfusion. Remarkably, no major changes in the rat brain proteome were noted after normothermic or hypothermic ischemia—even with durations as long as 30 min[46]. Reperfusion is likely to result in protein degradation via either calcium- or oxidation-mediated pathways. We also anticipate that reperfusion will also produce degradation of lipids. Both protein and lipid degradation may have a critical influence on the final outcome. To be able to address these pathways requires substantial molecular tools. Such tools are not available for use with tissue from dogs. In contrast, panoply of molecular tools is available for use with rat tissue. Thus, the ability to develop a rat model of SA was essential to allowing us to pursue these important questions—and is a current area of focus in our laboratory.

In our model, resuscitation from SA was first attempted using miniaturized CPB with small priming volume[47]. In pilot experiments, designed to mirror dog studies, we hypothesized that 30 min of SA would

be achievable in rats, and that Plasma-Lyte A would be a more favorable flush solution than normal saline. In our initial studies, HS was induced with rapid exsanguination (12.5 ml) over 5 min, followed by KCl-induced CA. After 2 min of no-flow, cooling was initiated with ice-cold flush and surface cooling. A target temperature of 10°C was chosen. After 30 min of SA, reperfusion and re-warming were achieved via CPB over 60 min. Four out of seven rats survived to 24 h at both groups. Favorable outcome (OPC 1) with minimal neurologic impairment on clinical assessment were achieved in both groups, with better results in Plasma-Lyte A group (4/7 rats) vs normal saline group (2/7 rats). Important to the potential future use of this model, microscopic alterations within the brain sections were minimal in the long-term survivors in these studies. Surprisingly, despite normal brain pathology, some extra-cerebral injury was noted on microscopic examination. Rats cooled with normal saline flush showed more severe heart, lung and kidney injury than those cooled with Plasma-Lyte A.

To our knowledge, this is one of the first descriptions of the successful use of CPB in a rat model that includes CA, and certainly the first study of the successful resuscitation of a rat *after* prolonged CA with deep or profound hypothermia. In our SA studies, the CA is induced before the induction of hypothermia. It is well recognized that it is much more challenging to successfully preserve or resuscitate an organism with hypothermia or any other strategy after the onset of a CA than it is to protect with hypothermia before the insult. All of our studies with SA have tackled the latter more challenging insult. Thus, it appears that SA is achievable in rats and can produce intact neurological outcome and normal brain histopathology. We are currently characterizing this model and pushing the 30 min limit that was achieved in our initial work.

Future studies focused at better organ preservation are underway in many centers. Diverse drugs such as PDE-4 inhibitor[48], xenon or other volatile anesthetics[49], P-selectin[43], and glutamine[45] have yielded favorable results in terms of ameliorating CPB-induced injury or ischemic injury, respectively. Other promising drugs including delta-opioid agonists or hibernation-induction triggers are emerging on the horizon. Knowledge of the molecular and cellular derangements will help target future therapies.

Successful establishment of this technically demanding model will allow the use of molecular tools to study effects of SA or deep hypothermia circulatory arrest and reperfusion on neuronal death and organ injury. This will have relevance to cardiac surgery and organ preservation. The ideal recipe for the SA-inducing flush solution remains to be determined. The optimal composition of this "magic potion" will, hopefully, be determined based on scientific evidence from our molecular studies—but may also require serendipity. Hemodynamic management must also be optimized during reperfusion, to maximize outcome.

Other Possible Applications of SA and the Futuristic Perspective of SA

In a broad sense, SA can be viewed as a strategy that bridges an organism over an insult that is otherwise incompatible with life. Besides traumatic CA, other potential targets for SA might include refractory normovolemic CA, cardiac or neurosurgical procedures that are impossible without extremely prolonged periods of circulatory arrest, consequences of chemical or biological warfare, and potentially other applications. When the milieu is improved by plasma exchange, administration of antidotes or the anatomic structure is corrected by surgery, delayed resuscitation can be started.

Conclusion

SA is a novel concept created to resuscitate traumatic CA. We have proven its feasibility in a series of large animal experiments using clinically-relevant SA models. The effectiveness of the hypothermic approach has been repeatedly demonstrated, and the maturity of the techniques prompts us to plan SA clinical trials with currently available devices in trauma centers in the setting of otherwise lethal exsanguination CA. To make SA eventually a technique for field resuscitation, further, bold, developments of cannulation and cooling techniques are also required. The exploration of pharmacologic approaches, however, needs more efforts to develop effective brain oriented preservation cocktails with drugs or solutions. Mechanistic studies using 2D proteomics and lipidomics may unveil the complex ischemic and reperfusion pathophysiology, and shed light into the area of pharmacological intervention.

We have taken the first steps toward moving the concept of SA from science fiction to reality—through rigorous scientific investigation. Further research into SA may not only lead to a substantial

improvement in the resuscitation of trauma-induced CA for first time in the past decades, benefit cardiovascular and neurological surgeries that need brain preservation techniques, and provide novel insight into the limits of viability of the brain and the entire organism during prolonged CA.

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Table 1 Studies of hypothermic approaches

CA time	Flush solution			Achieved/target	Outcome
	Volume	Approach	Avg. Efficiency (C/ml)	Tty*	
15 min	25 ml/kg (24°C)	intra-aortic balloon catheter		36a	
20 min	25ml/kg (24°C)	intra-aortic balloon catheter		35.7a	62(8-67) (n=6)
20 min	25 ml/kg (4°C)	intra-aortic balloon catheter	0.16	34a	5(0-49) (n=6)
30 min	25 ml/kg (4°C)	intra-aortic balloon catheter		34a	69(54-100) (n=6)
30 min	100ml/kg (4°C)	intra-aortic balloon catheter	0.10	28a	4(0-18) (n=7)
60 min	~159 ml/kg (4°C)	intra-aortic balloon catheter		20t	13 (0-27) (n=6)
60 min	~306 ml/kg (4°C)	intra-aortic balloon catheter	0.08	15t	0 (0-3) (n=5)
60 min	~469 ml/kg (4°C)	intra-aortic balloon catheter		10t	0 (0-0) (n=3)
90 min	~578 ml/kg (4°C)	intra-aortic balloon catheter	0.048	10t	0 (0-0) (n=6)
120 min	~666 ml/kg (4°C)	femoral artery catheter	0.046	7a	10 (0-39) (n=4)

*: a=achieved; t=targeted

Table 2 Exploration of pharmacological approaches for SA

	OPC 1	OPC 2	OPC 3	OPC 4	NDS (%)	HDS
Control	1/15	3/15	6/15	4/15	40 (18-63)	98 (58-136)
1. Energy metabolism						
Adenosine			2/2		50,43	116, 120
Thiopental	2/8		2/8	4/8	52 (22-57)	60 (52-138)
Thiop/Phenytoin	1/7		2/7	4/7	55 (38-59)	76 (48-132)
Fructose BiPhosphate			2/5	3/5	55 (39-63)	96 (76-102)
2. Membrane stability						
MK801			2/5	3/5	50 (33-55)	80 (41-109)
YM872			1/3	2/3	43,55,63	78,98,72
Nimodipine			1/2	1/2	33,66	86, 90
Diltiazem			1/2	1/2	47,64	134,100
Lidocaine			2/3	1/3	27,48,52	54,118,74
Insulin/Glucose	1/4		2/4	1/4	1,32,48,51	NA
3. Protein-kinase inhibitor						
W7*			1/2	1/2	66,48	108,98
4. Apoptosis inhibitor						
Cycloheximide			3/3		50,39,42	92,72,70
5. Antioxidant						
Tempo	5/8	3/8			9 (3-48)	58(35-78)
6. Mitochondria protection						
Cyclosporine A			1/2	1/2	41,71	NA

*:W7= *N*-(6-amino-hexyl)-5-chloro-1-naphthalenesulphonamide

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Figure Legend

Figure 1: Potential clinical scenario for application of suspended animation with delayed resuscitation as developed by investigators at the Safar Center for Resuscitation Research. See text for details.

(10). One can imagine clear-cut but rare scenarios where honoring this desire would be possible and straightforward. But Alzheimer's disease and other dementias often involve a long, slow decline. Patients may or may not have clearly articulated their preferences before cognitive impairment became too severe. Dr. Pisani and colleagues show that there are ample opportunities for patients with dementia to survive intercurrent episodes of critical illness, which presumably means they can wean from mechanical ventilation, recover from acute illness, and so on. But for how long?

It seems that those with dementia are a vulnerable population with special needs, not necessarily because of poor prognosis, as we now know from Dr. Pisani, but because we may not know their care preferences. Furthermore, with less than half of dementia cases being recognized by physicians, one can envision scenarios where decision-making ability is presumed, yet not present. Previously, it has been easy to assume, given a poor prognosis, that aggressive care would not be wanted—but what now? Should we manage these patients as if they would always want aggressive care? This is more aggressive than the care plan for patients without dementia—and, by extension, it likely flies in the face of Fried's findings. It would be easy in the early years to say "manage aggressively." But what about the subject who has lived with dementia for many years and has experienced several admissions and/or management crises for underlying medical conditions? When is it time to say, "enough is enough"? How can we try to help this process along, and what is the role of the intensivist? A good place to start might be to engage in ICU exit interviews to help families think about what they would do the next time their loved one becomes ill. Better yet would be to get patients better oriented to their potential future and more involved in advanced decision making well before dementia has become too advanced and the window of opportunity has closed.

This study has profound implications for patients with dementia, their families, and the physicians caring for them. Presumptions that these patients do not fare well with critical care should not drive treatment decisions. Instead, decisions regarding the use of life-sustaining therapies should be made only after considering the complete picture and, ideally, with an understanding of patient preferences.

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Should we add stem cells to the code cart in resuscitation of heatstroke?*

Stem cell researchers have shown promising breakthroughs that may ultimately enhance the quality of lives of victims who suffer devastating chronic diseases. However, because of the time needed for migration and differentiation by these cells, many scholars and scientists doubt if stem cells have any practical value to save lives in resuscitation. In resuscitation medicine and often in the intensive care unit, time is most critical. Either conventional or novel treatments delivered in a timely fashion are crucial to maximize outcome. Although the potential use of therapies such as with stem cells may have tremendous value across rehabilitation settings, with current technologies, resuscitation results are still critically limited by a very narrow time frame: an approximately 5-min no-flow duration in cardiopulmonary resuscitation, a "golden hour" for traumatic and hemorrhagic shock, and a possible 2-hr window in the treatment of heatstroke (1, 2). Presumably, this is not a race that one can win with cellular pseudopodia.

Although this assumption may have kept stem cells out of resuscitation medicine, in this issue of *Critical Care Medicine*, Dr. Chen et al. (3) revealed to us that stem cells—or, more precisely, a stem cell preparation—could surprisingly improve acute survival in an experimental animal model of heatstroke. Although the magnitude of the improvement in survival by the cell treatment is

*See also p. 1377.

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dwarfed by the findings of their prior studies using conventional drugs, such as dexamethasone (4) or an oral dose of a Chinese herbal compound (5), the study remains particularly interesting in that it may challenge the doubt about the value of stem cell preparations in acute resuscitation medicine.

Chen and his colleagues previously established special heatstroke models in rats (4–6). In spite of slight technical modifications and changes in the definition of "the onset of heatstroke" over the years, the models appear to be reproducible. They found that administration of human umbilical cord blood cells (HUCBCs) either intravenously or into the cerebral ventricle 68 mins after heat exposure significantly extended the survival time and preserved the brain histologically. In contrast, rats treated with vehicle died in approximately 22 mins, and brain histology revealed severe neuronal injury (3).

Treatment with peripheral blood mononuclear cells (PBMCs) also failed to produce any significant improvement. The authors attributed the discrepancy between PBMC and HUCBC treatments to the lack of pleuropotential of PBMCs (3).

Could the pleuropotential of HUCBCs be critical to this beneficial effect in resuscitation from heatstroke? We believe that cellular plasticity may be of limited importance in the current experimental paradigm. First, in such a short time (12 mins), it is inconceivable that there would be any functional differentiation, and if there were any, that it would affect hemodynamics or acute survival. Second, since no immunosuppression treatment was given, any functional and sustainable differentiation may not be possible with xenotransplantation, although this may not be a major issue in the acute situation. The speculation that the observed beneficial effects may be attributed to the changes in cytokines and nitric oxide production by the HUCBCs is more plausible (3). Indeed, something rapidly elaborated by the HUCBCs or released in response to their injection seems to be a likely mediator of these effects.

The biochemical and molecular mechanisms leading to improved survival and brain histologic findings with HUCBCs in the model need additional clarification. However, physiologically, it is appropriate to attribute the "neuroprotective effects" of HUCBC preparations to the hemodynamic improvement in the current study. Previously, this group has demonstrated increased lipid peroxidation in the brain during heat exposure (6), but more dramatic biochemical changes in the brain coincided with systemic hypotension after heat exposure was terminated. Therefore, brain injury is probably secondary to systemic circulatory collapse (5) or to a combination of hemodynamics and the direct central effect of hyperthermia. This assumption is also supported by a study of dogs by Oglesbee et al. (7) in which heat exposure at 42.5°C for 90 mins, which did not cause significant reduction in arterial blood pressure, did not produce any abnormalities in cerebral morphology or neurologic function after 8 days (7).

Although this study may be thought-provoking and define heretofore unrecognized potential hemodynamic effects of HUCBC preparations, there are several factors that may limit its implications.

First, many potential experimental artifacts may be operating. As stated in Methods, mean arterial pressure (MAP) decreased to 25 mm Hg at 68 ± 3 mins of heat exposure in the normal saline–treated group. As shown in Figure 1, however, MAP in both the HUCBC-treated and PBMC-treated groups was greater than 90 mm Hg at 68 mins of heat exposure, which suggests that these groups may have been less insulted than controls before cell injections. Similarly, the lack of full randomization of the study is a pitfall. In addition to these issues with study design, the findings of the neuropathological evaluation (60% of neurons were damaged over 12 mins) are rather unexpected. Neuronal damage generally takes time to mature and be seen with conventional histopathology. The findings in many animal studies suggested that the acute central nervous injuries may be very subtle, even in animals with lethal heatstroke (8). Few previous studies had examined the short-term histologic effects on the brain immediately after heat exposure (8), and the differences in heatstroke models confound the interpretation of their brain histologic findings.

Second, as a general principle, novel drugs/compounds should not be tested in an "underresuscitated" model. Otherwise, one might target a problem that actually is not a clinical concern, or the effectiveness of the experimental treatments becomes ineffective when conventional treatments are provided. Unfortunately, this principle was not followed in the current study. Clinically, the conventional treatment of heatstroke (systemic hyperthermia) includes aggressive cooling and support of vital organs (1, 2, 9). Central nervous system functions are disturbed, but the brain is not the first organ at risk (8). If circulatory, hepatic, and renal dysfunction and hematologic disorders are prevented, brain injury can often be avoided.

In summary, there may be some beneficial ingredients in the HUCBC preparation, but it remains to be determined whether this must be delivered with intact cells. Local versus systemic delivery is also a logistic concern. Finally, it is important to note that cooling and support of extracerebral organs are critical in the initial treatment of heatstroke. Although this study by Chen et al. is provocative and stem cell therapies may have great promise in the rehabilitation of central nervous system injury, it is unclear whether they will ever find a place in the code cart for resuscitation.

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ABSTRACT

Introduction: Clinical studies have demonstrated improved survival after cardiac arrest with induction of mild hypothermia (34°C). Infusion of ice-cold saline seems beneficial. The American Heart Association recommends therapeutic hypothermia for comatose survivors of cardiac arrest. For hemorrhagic shock (HS), laboratory studies suggest that mild hypothermia prolongs the golden hour for resuscitation. Yet, the effects of hypothermia during HS are unclear since retrospective clinical studies suggest that hypothermia is associated with increased mortality. Using a clinically relevant, large animal model with trauma and intensive care, we tested the hypothesis that mild hypothermia, induced with intravenous cold saline (ice cold or room temperature) and surface cooling, would improve survival after HS in pigs.

Methods: Pigs were prepared under isoflurane anesthesia. After laparotomy, venous blood (75 ml/kg) was continuously withdrawn over 3 h (no systemic heparin). At HS 35 min, the spleen was transected. At HS 40 min, pigs were divided into 3 groups (n=8, each): 1) Normothermia (Norm)(38°C) with warmed saline, 2) Mild hypothermia (34°C) induced with i.v. infusion of 2°C saline (Hypo-Ice) and surface cooling, and 3) Mild hypothermia (34°C) with room temperature (24°C) i.v. saline (Hypo-Rm) and surface cooling. Fluids were given when mean arterial pressure (MAP) was <30 mmHg. At HS 3 h, shed blood was returned and splenectomy was performed. Intensive care was continued to 24 h.

Results: At 24 h, there were 2 survivors in the Norm Group, 4 in the Hypo-Ice Group and 7 in the Hypo-Rm Group ($p<0.05$ vs the Norm Group, Log Rank). Time required to achieve 34°C was 17±9 min in the Hypo-Ice Group and 15±4 min in the Hypo-Rm Group (NS). Compared to the Hypo-Rm Group, the Hypo-Ice Group required less saline during early HS (321±122 vs

571±184 ml, $p<0.05$). The Hypo-Ice Group also had higher lactate levels than the Hypo-Rm Group ($p<0.05$). Hypothermia did not cause any increase in bleeding compared to normothermia.

Conclusion: Mild hypothermia during HS, induced by infusion of room temperature saline and surface cooling, improves survival in a clinically relevant model of HS and trauma. However, rapid administration of ice-cold resuscitation fluid may have detrimental effects during HS, possibly due to induced vasoconstriction. These findings suggest that optimal methods for induction of hypothermia need to be addressed for each potential indication, e.g., cardiac arrest vs HS.

INTRODUCTION

After cardiac arrest, 2 large, randomized, controlled clinical trials[1,2] demonstrated that induction of mild hypothermia (33-34°C) in comatose survivors of cardiac arrest could improve survival and neurologic dysfunction. Based on these findings, the American Heart Association and the International Liaison Committee on Resuscitation recommended cooling all cardiac arrest victims who remain comatose to 32-34°C for 12-24 h.[3]

The effect of hypothermia on survival from trauma and hemorrhagic shock (HS) is much more controversial. Clinical retrospective analyses have suggested that hypothermia is associated with poor outcome in trauma patients. [4,5] This supports the recommendations of the Advanced Trauma Life Support Course[6] that hypothermia should be avoided in trauma patients. In contrast with the clinical data, studies in experimental models have consistently showed that mild hypothermia improves survival during and after volume-controlled, [7,8] pressure-controlled,[9] and uncontrolled HS.[10,11] To understand these differences, it is critical to consider the difference between secondary hypothermia (from exposure, administration of cold fluids, shock, anesthetics, etc) and therapeutic, induced hypothermia with prevention of shivering and sympathetic response by sedatives, anesthetics and/or muscle relaxants and with rigorous control of target temperature.[12]

Previous laboratory studies, however, have not included significant tissue trauma. Similarly, studies in clinically relevant large animal models with prolonged intensive care are lacking. Therefore, to explore the effects of mild hypothermia using a more clinically-relevant insult and resuscitation, we established a large animal HS model with trauma via laparotomy and splenectomy followed by intensive care life support to 24 h.

Although a recent clinical study in patients after cardiac arrest showed that hypothermia remained effective even if patients were cooled to the target temperature over 8 h,[2] it is generally believed that the faster hypothermia is induced, the more effective it will be. Kuboyama *et al* reported that cooling initiated 15 min after return of spontaneous circulation had almost no effect in a canine cardiac arrest model.[13] Ice-cold lactated Ringer's solution has recently been used after cardiopulmonary resuscitation (CPR) to facilitate induction of hypothermia. Infusing 30 ml/kg 4°C saline over 30 min rapidly after resuscitation from cardiac arrest decreased the body temperature by 1.7°C in 20 patients and increased blood pressure.[14] No significant side effects, such as arrhythmia or pulmonary edema were found. Based on the efficacy of mild hypothermia in animal models of HS, it is logical to assume that ice-cold saline, if well tolerated, may be an ideal solution for both induction of hypothermia and volume replacement during initial resuscitation from HS. Indeed, Norio *et al* reported that infusion of a fixed volume (500 ml) of 4°C saline over 20 min decreased the core temperature by 2°C, and significantly prolonged the short-term survival time in pigs with uncontrolled bleeding from aortomy.[15] Rapid induction of mild hypothermia has not, however, been studied in a large animal model of HS with assessment of long-term outcome.

In the current study, we developed a clinically-relevant HS model in pigs with the following features: 1) controlled continuous hemorrhage during HS simulating uncontrolled bleeding over 3 h; 2) laparotomy and spleen transection; 3) limited fluid resuscitation (simulating field conditions), and 4) subsequent full resuscitation and 24 h life support. Normothermia was compared to mild hypothermia (34°C) (induced with surface cooling and intravenous infusion of either room temperature or ice-cold saline during HS) in terms of physiologic parameters, survival time, and long- term survival rate.

We hypothesized that, compared to maintenance of normothermia, mild hypothermia would improve survival from prolonged, traumatic HS in a clinically relevant large animal model with intensive care life support. We also hypothesized that infusion of ice-cold saline during resuscitation would facilitate cooling and further improve survival.

MATERIALS AND METHODS

This study was approved by the Institutional Animal Care and Use Committee of the University of Pittsburgh.

Design of the Study [Fig 1]

Thirty-one Yorkshire/Landrace crossbred swine (Whippo Farms Enon Valley, PA), age 9-11 weeks, weighing 20.0-33.2 kg were used. The HS model incorporated continuous stepwise bleeding via a catheter placed in the right external jugular vein. The hemorrhage phase lasted 3 h. At HS 35 min, the spleen was transected. At HS 40 min, limited (hypotensive) fluid resuscitation was started, simulating arrival of paramedics. Pigs were randomized into 3 groups: 1) Normothermia (38°C) with warmed saline (Norm), 2) Hypothermia (34°C) induced with infusion of 2°C saline and surface cooling (Hypo-Ice), and 3) Hypothermia (34°C) with room temperature (24°C) saline and surface cooling (Hypo-Rm). Resuscitation fluids were given when MAP was <30 mmHg. After the target temperatures were achieved, the resuscitation fluid was switched to warmed lactated Ringer's (LR, standard resuscitation fluid) to prevent temperature overshoot. At HS 3 h, shed blood was returned, and splenectomy was performed. Life support and intensive care were continued to 24 h.

Anesthesia and Surgical Preparation

The pigs were fasted overnight with free access to water. They were premedicated with intramuscular injection of ketamine 20 mg/kg, xylazin 2 mg/kg, and atropine 0.5 mg. Following administration of 1.5- 2% isoflurane via a cone mask for 5 min, endotracheal intubation was performed, and mechanical ventilation was started with a tidal volume of 10 ml/kg and a frequency of 14-16 breaths/min (Piston Ventilator Model 613, Harvard Apparatus, South Natick, MA), adjusted to maintain an arterial PCO_2 of 35-45 mmHg. An 18-G cannula (Angiocath,

Becton Dickinson, Infusion Therapy Systems Inc. Sandy, Utah) was inserted into the ear vein for fluid infusion. The maintenance fluid (0.45% NaCl in 5% Dextrose) was infused at a rate of 4 ml/kg/h to assure normovolemia and normoglycemia. Electrocardiogram (EKG) was continuously monitored with standard lead II.

A sterile cutdown was performed in the right groin, and a PE 90 catheter was inserted into the right femoral artery for blood pressure monitoring. Through a sterile cutdown on the right side of the neck, a 16 G catheter was inserted 20 cm into the external jugular vein for continuous blood withdrawal. A PE 60 catheter was inserted into the lumen of the blood withdrawal catheter for infusion of citrate solution during blood withdrawal for maintenance of catheter patency and preservation of shed blood. The tip of the citrate catheter was 3 cm from the tip of the blood withdrawal catheter. A pulmonary artery catheter was inserted via the right cephalic vein into the inferior vena cava to measure the core body temperature without contamination from the resuscitation fluid administered via the superior vena cava system. A sterile cutdown was performed on the left side of the neck and a PE 90 catheter was inserted into the cephalic vein for fluid infusion. A cystostomy was performed through a sterile 5 cm lower midline laparotomy, and a balloon catheter was placed for urine drainage. Esophageal and rectal temperatures were all monitored.

Core temperature (blood temperature at the inferior vena cava) was maintained at 38.0°C with heating blankets and heating lamps prior to the insult.

Twenty min was allowed for stabilization before baseline hemodynamics and blood samples were collected. Group randomization was performed during preparation.

Laparotomy and Induction of HS

A sterile 12 cm midline laparotomy was performed and the spleen was gently exposed for transection at HS 35 min. The wound was temporarily closed with towel clips.

Just prior to induction of HS, the heating blankets and lamps were turned off and fluid infusion was stopped. The FiO_2 was set at 0.25 with 75% N_2. The isoflurane was kept at the baseline level. It was decreased to 1% when MAP was 50 mmHg, to 0.5% at MAP 40 mmHg, and to 0.2% at MAP less than 15 mmHg. Starting from HS 40 min, isoflurane was set at 0.5%. Pancuronium 0.2 mg/kg was given at the beginning of HS and repeated as needed. Pancuronium was avoided in the late HS phase (HS >1.5 h) because its adverse circulatory effects[16,17] may artificially shorten the survival time.

Venous blood withdrawal was controlled in a continuous and graded fashion: 1) 68.6 ml/kg/h for the first 35 min, 2) 20 ml/kg/h from HS 35-95 min, and 3) 10 ml/kg/h from HS 95-180 min. At HS 35 min, the spleen was transected at the mid point. The splenectomy was delayed until the animals were hypotensive to avoid large amounts of blood loss that could not be replaced without the availability of a blood bank during resuscitation. The transected spleen remained in the abdomen, untouched until the end of HS.

Pigs that developed cardiac arrest (ventricular fibrillation, pulseless electrical activity, or asystole) prior to 40 min were excluded from further evaluation. The pigs were divided into 3 groups for temperature control after HS 40 min: 1) Norm, in which the core temperature was maintained at 38.0±0.5°C. Warmed (38°C) saline was infused when MAP decreased below 30 mmHg for limited fluid resuscitation. 2) Hypo-Ice, in which the core temperature was decreased to 34°C with surface cooling. Ice-cold (2°C) saline was used for limited fluid resuscitation. 3) Hypo-Rm, similar to Group Hypo-Ice except that room temperature (20°C) saline was utilized.

When the respective target temperatures were achieved, warmed LR was used for hypotensive fluid resuscitation if needed.

Resuscitation and Splenectomy

At HS 180 min, the shed blood from the first 30 min was reinfused. Additional LR was given to restore MAP >70 mmHg. The splenectomy was performed and the abdominal wound was closed. The quantity of blood loss from the spleen was measured.

Intensive Care

Analgesia and mechanical ventilation. Morphine 15 mg IV was administered for signs of distress (midriasis, tachycardia, movement). Pancuronium 0.2 mg/kg was administered to facilitate mechanical ventilation after sufficient opiate was given.

Temperature. The temperature in the hypothermia groups was maintained at 34°C until resuscitation time (RT) 12 h, followed by a slow rewarming (1°C/h) to 38°C. Group Norm was maintained at 38°C throughout the experiment.

Hemodynamics and Fluid balances. LR was administered when MAP was less than 70 mmHg and CVP was less than 8 mmHg. Infusion of norepinephrine was started when hypotension was accompanied by an elevated CVP (>8 mmHg).

Electrolytes and acid-base balance. Potassium chloride (20 mEq) was added to the maintenance fluid when plasma potassium was lower than 3 mmol/L. If plasma calcium was less than 1 mmol/L, 200 mg $CaCl_2$ was slowly injected. If base deficit (BD) was greater than 6 mmol/L, sodium bicarbonate ($NaHCO_3$) (bodyweight x BD/6) was administered.

At 24 h, all pigs were euthanized with an overdose of isoflurane and KCl. A thorough necropsy was performed.

Statistic Analysis

Data are presented as mean ± standard deviation unless otherwise stated. During HS 40-60 min (the time for cooling in the hypothermic groups), the requirements for fluids were analyzed with analysis of variance (ANOVA) followed by Tukey-Kramer post-hoc test. Changes in MAP during induction of hypothermia (HS 40-60 min) were analyzed separately using one way ANOVA for repeated measurements. The Student t-test was used in the analysis of arterial lactate levels at HS 2 h and 3 h in the two hypothermia groups, because there were only 2 surviving pigs in the Norm Group at HS 2 and 3 h. The survival rates were analyzed with Fisher's exact test, and the survival time using the Log-Rank life table analysis.

RESULTS

Of the 31 pigs initially studied, 7 (23%) died before HS 40 min and were excluded from analysis. Their survival time was median of 39 min (range: 35-40 min).

There were no significant differences in the baseline variables amongst the 3 groups (Table 1).

Hemorrhagic shock phase

Hemodynamics. Hemodynamics and physiological parameters were not different between groups before randomization at HS 40 min (Table 1). After HS 40 min, MAP was not significantly different between groups (Fig. 2). The heart rates were significantly lower in both hypothermia groups ($p<0.01$, vs Norm Group) (Fig 3). The amount of resuscitation fluid required during induction of hypothermia was 507±93 ml in the Norm Group, 571±184 ml in the Hypo-Rm Group, and 321±122 ml in the Hypo-Ice Group ($p<0.05$, Hypo-Ice Group vs Hypo-Rm Group). The total fluid requirement over 3 h of HS was not significantly different between groups. The total blood loss from the spleen was <30 ml in all pigs.

Temperatures. Core temperature increased slightly during the initial HS phase (HS 0-40 min). The temperature in the Norm Group was then maintained at 37.5-38.5°C throughout the remaining HS phase. Time to reach 34°C was 17±9 min in the Hypo-Ice Group and 15±4 min in the Hypo-Rm Group (NS) (Fig. 4).

Physiologic parameters. There were no significant differences in pH, PCO_2, BD, hematocrit, glucose or plasma potassium levels at HS 1, 2 or 3 h. At HS 2 and 3 h, the lactate levels in the Hypo-Ice Group were significantly higher than the Hypo-Rm Group ($p<0.05$) (Table 1).

Resuscitation and ICU phase

Two of 8 pigs in the Norm Group survived the HS phase, compared to 5 of 8 in the Hypo-Ice Group and 7 of 8 in the Hypo-Rm Group. There were no significant differences in MAP (Fig. 2) or HR (Fig. 3) amongst groups. The lactate levels were higher in the Hypo-Ice Group vs the Hypo-Rm Group during the early resuscitation phase; however, lactate levels in both groups rapidly fell into the normal range. The remaining physiologic parameters were not significantly different between groups (Table 1).

One pig in the Hypo-Ice Group died at RT 10 min. One pig in the Hypo-Rm Group died at RT 12 h of a tension pneumothorax caused by a ventilator malfunction. The pig had stable hemodynamics and normal blood gas and lactate levels up to that point and most likely would have survived to 24 h. At necropsy there was no gross organ damage.

Final outcome

Survival to RT 24 h was achieved by 6 of 8 Hypo-Rm pigs (1 animal in the Hypo-Rm Group was censored at 12 h), 4 of 8 Hypo-Ice pigs, and 2 of 8 Norm pigs ($p=0.01$, Hypo-Rm vs Norm) The survival time (Fig. 5) was significantly longer in Group Hypo-Rm compared to Group Norm ($p<0.01$).

At 24 h, there were no significant differences in cardiac enzymes, liver injury tests, or renal function between groups (Table 2).

At necropsy, there was no gross organ damage found in any survivors.

DISCUSSION

This study demonstrated that mild hypothermia improves survival in a clinically relevant pig HS model that includes significant tissue trauma, as well as resuscitation and intensive care similar to clinical situations. This is an important first. In addition, we found that induction of hypothermia using ice-cold fluid may have back-fired, since use of room temperature fluid lead to a similar cooling rate with improved survival.

The finding that mild hypothermia improves survival from HS corroborates our previous hypothermia studies in rat HS models, which consistently demonstrated that hypothermia, induced either during HS or during fluid resuscitation, was associated with improved survival compared to normothermic resuscitation.[8,9,11,18] Other investigators also reported various beneficial effects of hypothermia during HS in animal experiments.[19-22] The hemorrhage insult in this study was very severe. Twenty-three percent of all subjects died before HS 40 min. In a previous rat study[23], in which we allowed spontaneous cooling to occur during HS, continuing mild hypothermia during resuscitation improved survival compared to active rewarming, particularly with a more severe, highly lethal insult. Deaths in these studies occurred relatively early during resuscitation and intensive care, with cardiovascular collapse unresponsive to aggressive resuscitation, rather than delayed multiple organ system failure. Meyer and Horton[21,22] had previously demonstrated that mild hypothermia during HS improves cardiac performance compared to maintenance of normothermia in dogs. Similarly, Mizushima, et al[24], found that cooling to 32°C during HS in rats preserved cardiac performance better than maintenance of normothermia. Prolonged hypothermia at this level seemed to be detrimental, however. Thus it seems that the main benefit of hypothermia during HS and resuscitation may be

protective effects on the heart and peripheral vasculature, though this will need further study. These results may indicate that those with very severe HS may benefit most from hypothermia.

Our HS model with continuous, controlled bleeding in pigs was established based on rat models of Healey et al[25] and Alam et al[26]. The model was designed to study prolonged hypotensive resuscitation, simulating medical support when transportation or evacuation is delayed as in rural and military settings. Using large animals facilitated the inclusion of clinically relevant components including avoidance of systematic heparin, addition of tissue trauma (laparotomy and spleen transection) and controlled life support during the post-resuscitation phase.

One surprising finding in this study was that the method of induction of hypothermia with IV fluids produced markedly different physiologic effects and impact on survival, despite similar temperature curves. The fact that the infusion of ice-cold fluid was associated with increased MAP and blood lactate in the Hypo-Ice Group suggests that vasoconstriction with decreased tissue perfusion may have played a role in worsening outcome. Two questions then arise. First, could ice-cold saline further stimulate a cold response, such as vasoconstriction, after animals had already been in shock and had been fully exposed to surface cooling? Second, is the vasoconstriction effect responsible for the worsened outcome?

Ice-cold saline is a very potent stimulus to the thermoregulation center. Infusion of 40 ml/kg of either 4°C or 20°C saline to awake human volunteers decreased the core temperatures by 2.5 or 1.4°C, respectively.[27] Infusion of either 30 or 60 ml/kg cold (4°C) saline over 30 min increased plasma norepinephrine by 220 to 700 %,[28,29] while epinephrine levels remained unchanged until a threshold (decrease in core temperature of 1°C) was reached.[29] Cheng et al[30] found that changes in the surface temperature contributed only about 20% to the cold response.

Therefore, it seems that changes in core temperatures more readily trigger cold responses, such as vasoconstriction and shivering. Based on the above evidence, it is possible that the Hypo-Ice Group had a more potent cold stimulus resulting from a lower temperature of resuscitation solution.

Among the major cold responses, shivering and vasoconstriction are likely to affect the course of HS. Because of the use of muscle relaxants in this study, shivering was prevented, leaving vasoconstriction as the major cold-induced physiological response. Since the 1950s when Close at al[31] demonstrated that infusion of norepinephrine during HS increased mortality in a dog model, vasoconstriction is believed to be detrimental for resuscitation from HS. However, physiologically, vasoconstriction is a life-saving response that redistributes limited blood flow to vital organs after trauma and hemorrhage. In certain situations, exogenous vasopressors are complementary to the insufficient or exhausted physiological vasoconstriction. Recently, it has been reported that vasoconstriction induced pharmacologically during HS appeared to improve acute survival.[32-34] Morales et al[35] reported that infusion of vasopressin to dogs that were not responsive to norepinephrine late in HS dramatically increased arterial blood pressure. In general, however, the enhanced vasoconstriction induced by drugs or hypothermia is a double edge sword. Accompanying improved acute survival, Alspaugh *et al*[33] found a marked increase in brain lactate/pyruvate ratio, a marker of adequate perfusion that was determined by microdialysis, when animals were treated with a vasopressor during HS.

Studies of volume-controlled or uncontrolled HS have shown that hypothermia increases MAP during shock.[8,10] However, with less severe HS than studied here, we previously reported that controlling for blood pressure during HS did not negate the beneficial effects of hypothermia.[9]

Another possible explanation for the lack of benefit of the ice-cold fluid may have been related to specific characteristics of the model itself. The volume of fluid administered during HS was dependent upon MAP. The Hypo-Ice group received less fluid during the early phase of limited fluid resuscitation since the MAP increased quickly, likely because of the impact of hypothermia on MAP. This question deserves further study.

In addition to the above vascular effects of ice-cold saline, the possible cardiac effects of ice-cold saline deserve additional attention. In our HS model, most pigs had a MAP around 25 mmHg (range 12-32 mmHg) immediately before the start of limited fluid resuscitation. When fluid was given at the rate of ~50 ml/min, MAP usually increased steadily. However, one pig in the Hypo-Ice Group had a MAP of only 13 mmHg at HS 40 min, accompanied by a decelerating HR from 200 bpm at HS 35 min to 165 bpm. Within 2 min of the rapid infusion of ice-cold saline asystole occurred. We have noticed that deceleration of heart rate is a sign of decompensation, often followed, within a few minutes, by severe bradycardia and cardiac arrest. The rapid infusion of ice-cold fluid during profound hypovolemic hypotension may risk causing cardiac arrest. This has not been observed after normovolemic cardiac arrest in humans[14] and suggests important potential differences in the response to rapid cooling in HS vs normovolemic cardiac arrest.

One rationale for infusion of ice-cold saline is the belief that the faster hypothermia is induced, the more effective it will be. The need for ice-cold fluid to increase the rate of cooling may be less of an issue during HS than it is after cardiac arrest. First, patients in HS tend to become hypothermic spontaneously because of exposure and administration of room temperature fluids, as well as a decreased ability to maintain normothermia because of shock itself, anesthetics/sedatives, alcohol and drug use. Second, in contrast to normovolemic cardiac arrest,[14]

victims of HS need copious amounts of fluid for resuscitation, which, if stored at room temperature, will add to the cooling.[36] Third, we have found that hypothermia has similar benefits on survival when induced at 10 min or 1 h of HS in rats;[37] thus, the therapeutic window may be substantial.

Our study is limited in several ways. First, unlike the work of Norio et al[15], we did not use a fixed volume of ice-cold saline in the resuscitation protocol. Fixed volume resuscitation regardless of response may be relevant in military or certain civilian pre-hospital settings. However, ideally, resuscitation should always be guided by certain endpoints. In our study, we titrated fluid administration to MAP, which is important in order to avoid increased bleeding and mortality.[38]

The model is different from clinical reality for a number of reasons. Use of anesthesia, muscle relaxants, endotracheal intubation and mechanical ventilation before and during HS are necessary, but do not mimic the clinical condition. However, at the time of induction of hypothermia, it is important to blunt the shivering and sympathetic responses to hypothermia by making the organism poikilothermic using anesthetics and muscle relaxants. Takasu et al[39] found that surface cooling during severe HS without any fluid resuscitation actually was ineffective at decreasing core temperature and actually shortened the survival time in pigs under light halothane anesthesia.

The laparotomy was performed prior to the splenic injury out of necessity. Obviously, in the clinical situation, the trauma to the spleen would have occurred first. We did perform the laparotomy as close as possible to the onset of hemorrhage.

Another issue with the model, though not specific to hypothermia, is the use of whole, autologous blood for transfusion during resuscitation. Clinically, the effects of donated blood

that has been separated into packed cells and other components, and then stored for a prolonged period of time, are significant.

One of the biggest issues in hypothermic trauma patients is coagulopathy. This may be one of the critical reasons for the discrepancy between the clinical and laboratory findings related to hypothermia and HS/trauma. In this study and a previous one in pigs with cooling to 34°C,[40] we did not observe clinically significant bleeding from the liver or transected spleen during HS. Even in clinical studies, tests of the coagulation system and platelet function do not demonstrate significant changes until temperature is reduced to below 34°C.[41] The fact that cooling head injured patients did not cause any increase in intra-cranial hemorrhage also demonstrates this point.[42]

In conclusion, in a model of continuous bleeding in pigs, we found that mild hypothermia (34°C) induced with surface cooling and infusion of room temperature fluids during HS improved survival. In contrast, infusion of ice-cold saline may induce excessive vasoconstriction or cause cardiac arrest, undermining hypothermia's beneficial effects. Clinical safety and feasibility studies of mild hypothermia for resuscitation of trauma victims with evidence of HS should be conducted, followed by randomized clinical trials.

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Table 1. Physiologic data during HS and resuscitation

	Group	BL	HS 40 min	HS 1h	HS 3h	RT 6h	RT 24h
pH	Norm	7.58±0.04	7.56±0.05	7.47±0.05	7.13±0.01	7.51, 7.54	7.54, 7.54
	Hypo-Ice	7.57±0.04	7.55±0.06	7.45±0.05	7.20±0.08	7.4±0.0	7.5±0.1
	Hypo-Rm	7.55±0.02	7.50±0.04	7.38±0.05	7.24±0.06	7.4±0.1	7.5±0.0
PCO_2 (torr)	Norm	34.0±5.4	24.0±7.6	26.4±5.8	27.0±1.8	35.7, 32.5	30.7, 36.2
	Hypo-Ice	35.8±3.5	22.1±5.8	27.6±5.8	28.2±4.6	40.8±3.3	37.1±3.1
	Hypo-Rm	35.4±4.3	25.1±5.3	32.4±3.8	29.0±3.1	41.3±3.4	34.5±3.0
PO_2 (torr)	Norm	206±25	92±21	103±20	170±55	183, 93	182, 138
	Hypo-Ice	199±26	112±15	132±18	191±53	201±71	209±47
	Hypo-Rm	205±23	111±16	133±14	175±45	230±77	263±24
Base deficit (mmol/L)	Norm	-10.6±3.1	-0.3±4.3	2.9±5.1	17.5±1.1	-6.2, -3.1	-4.8, -8.4
	Hypo-Ice	-10.9±2.9	1.6±2.9	3.1±4.0	15.0±2.0	-2.7±2.3	-8.4±2.3
	Hypo-Rm	-9.0±2.5	1.9±3.5	4.2±3.7	12.8±1.6	-3.2±3.7	-7.8±3.0
Glucose (mg/dl)	Norm	118±29	184±116	181±90	319	338, 153	132, 92
	Hypo-Ice	125±26	200±117	160±82	397±59	309±81	103±15
	Hypo-Rm	125±30	170±90	209±73	511±110	391±116	126±43
SvO_2 (%)	Norm	79.6±6.0	9.4±3.9	13.6±9.3	21.4,10.2	81.2, 79.0	82.8, 84.0
	Hypo-Ice	78.8±4.7	19.2±9.8	21.4±18.8	15	79.4±11.9	79.8±5.3
	Hypo-Rm	75.0±8.7	19.3±9.8	25.3±18.2	25.1±12.9	77.8±8.0	82.4±8.4
Lactate (mmol/L)	Norm	1.2±0.6	6.9±1.6	9.0±2.2	16.5	2.7, 1.7	0.5, 1.0
	Hypo-Ice	0.8±0.4	7.2±1.1	8.4±1.4	18.4±2.3*	5.3±3.0	0.7±0.3
	Hypo-Rm	0.5±0.3	6.3±1.2	7.6±1.9	15.2±1.3	4.2±1.8	0.4±0.4
Hematocrit (%)	Norm	29.3±1.8	28.5±3.2	24.1±4.1	11.0,10.0	26, 26	29, 23
	Hypo-Ice	30.0±1.9	29.9±4.1	26.0±4.5	9.7±2.5	33.3±2.4	26.3±2.8
	Hypo-Rm	29.4±2.7	28.8±4.8	21.4±3.2	11.5±2.3	34.3±3.3	27.8±2.2

Data are presented as mean ±standard deviation. Individual data are presented if n<3. BL=baseline, HS=hemorrhagic shock, RT=resuscitation time, SvO2=Oxygen saturation of central venous blood. *: $p<0.05$, vs Group Hypo-Rm

Table 2. Cardiac, hepatic and renal damage in survivors

	Group	Baseline	RT 24 h
Bilirubin (mg/dl)	Hypo-Ice	<0.1	<0.1
	Hypo-Rm	<0.1	<0.1
Aspartate aminotransferase (IU/L)	Hypo-Ice	32±5	67±11
	Hypo-Rm	34±7	108±36
Alanine aminotransferase (IU/L)	Hypo-Ice	63±20	71±20
	Hypo-Rm	54±14	69±12
γ-glutamyl transpeptidase (IU/L)	Hypo-Ice	45±21	44±16
	Hypo-Rm	36±19	41±28
Alkaline phosphatase (IU/L)	Hypo-Ice	171±40	193±79
	Hypo-Rm	139±26	156±37
Troponin-I (ng/mL)	Hypo-Ice	0.51, 0.76	4.9, 18.1
	Hypo-Rm	0.41±0.18	18.76±12.72
Creatinine (mg/dL)	Hypo-Ice	1.2±0.1	1.1±0.1
	Hypo-Rm	1.2±0.3	1.7±08

Data are presented as mean±standard deviation. N=3 in Hypo-Ice Group, and N=6 in Hypo-Rm Group. Only 2 animals had values for troponin in the Hypo-Ice group. RT=Resuscitation time

FIGURE LEGENDS

Figure 1
Experimental protocol. LR=lactated Ringer's; MAP=mean arterial pressure; FR=fluid resuscitation.

Figure 2
Mean arterial pressure (MAP) during hemorrhagic shock (HS) and early resuscitation. Groups are normothermia (Norm), mild hypothermia with ice-cold flush (Hypo-Ice), and mild hypothermia with room temperature flush (Hypo-Rm).

Figure 3
Heart rate (HR) during hemorrhagic shock (HS) and early resuscitation. Groups are normothermia (Norm), mild hypothermia with ice-cold flush (Hypo-Ice), and mild hypothermia with room temperature flush (Hypo-Rm).

Figure 4
Core temperature during hemorrhagic shock (HS) and early resuscitation. Groups are normothermia (Norm), mild hypothermia with ice-cold flush (Hypo-Ice), and mild hypothermia with room temperature flush (Hypo-Rm).

Figure 5
Survival following prolonged hemorrhagic shock and resuscitation. Groups are normothermia (Norm), mild hypothermia with ice-cold flush (Hypo-Ice), and mild hypothermia with room temperature flush (Hypo-Rm). *One pig in the Hypo-Rm group died of a tension pneumothorax at 12 h.

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Figure 1

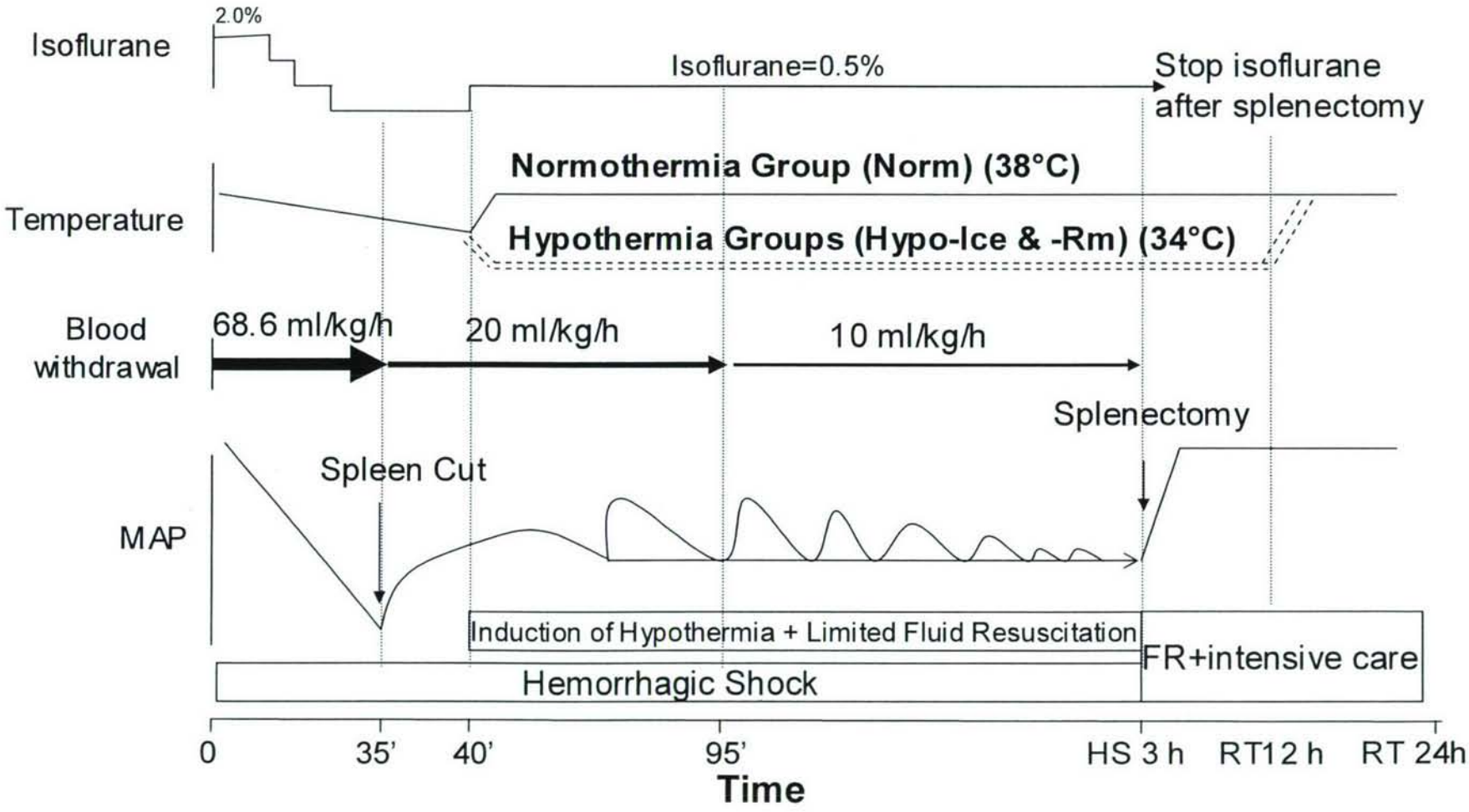

Isoflurane
2.0%
Isoflurane=0.5%
Stop isoflurane after splenectomy
Normothermia Group (Norm) (38°C)
Temperature
Hypothermia Groups (Hypo-Ice & -Rm) (34°C)
Blood withdrawal
68.6 ml/kg/h
20 ml/kg/h
10 ml/kg/h
Splenectomy
Spleen Cut
MAP
Induction of Hypothermia + Limited Fluid Resuscitation
FR+intensive care
Hemorrhagic Shock
0
35'
40'
95'
HS 3 h
RT12 h
RT 24h
Time

Figure 2

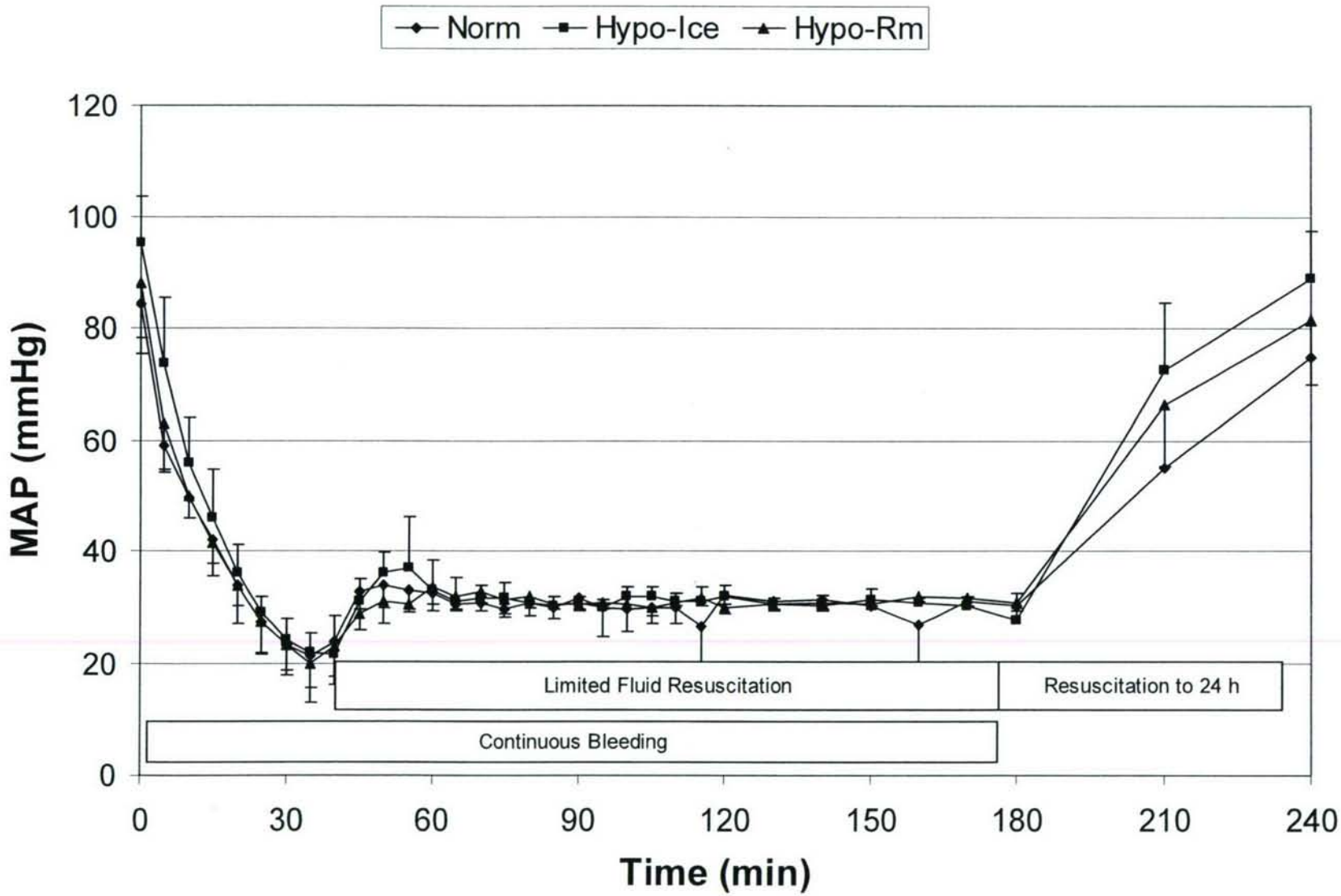

Norm
Hypo-Ice
Hypo-Rm
MAP (mmHg)
120
100
80
60
40
20
0
0
30
60
90
120
150
180
210
240
Time (min)
Limited Fluid Resuscitation
Resuscitation to 24 h
Continuous Bleeding

Figure 3

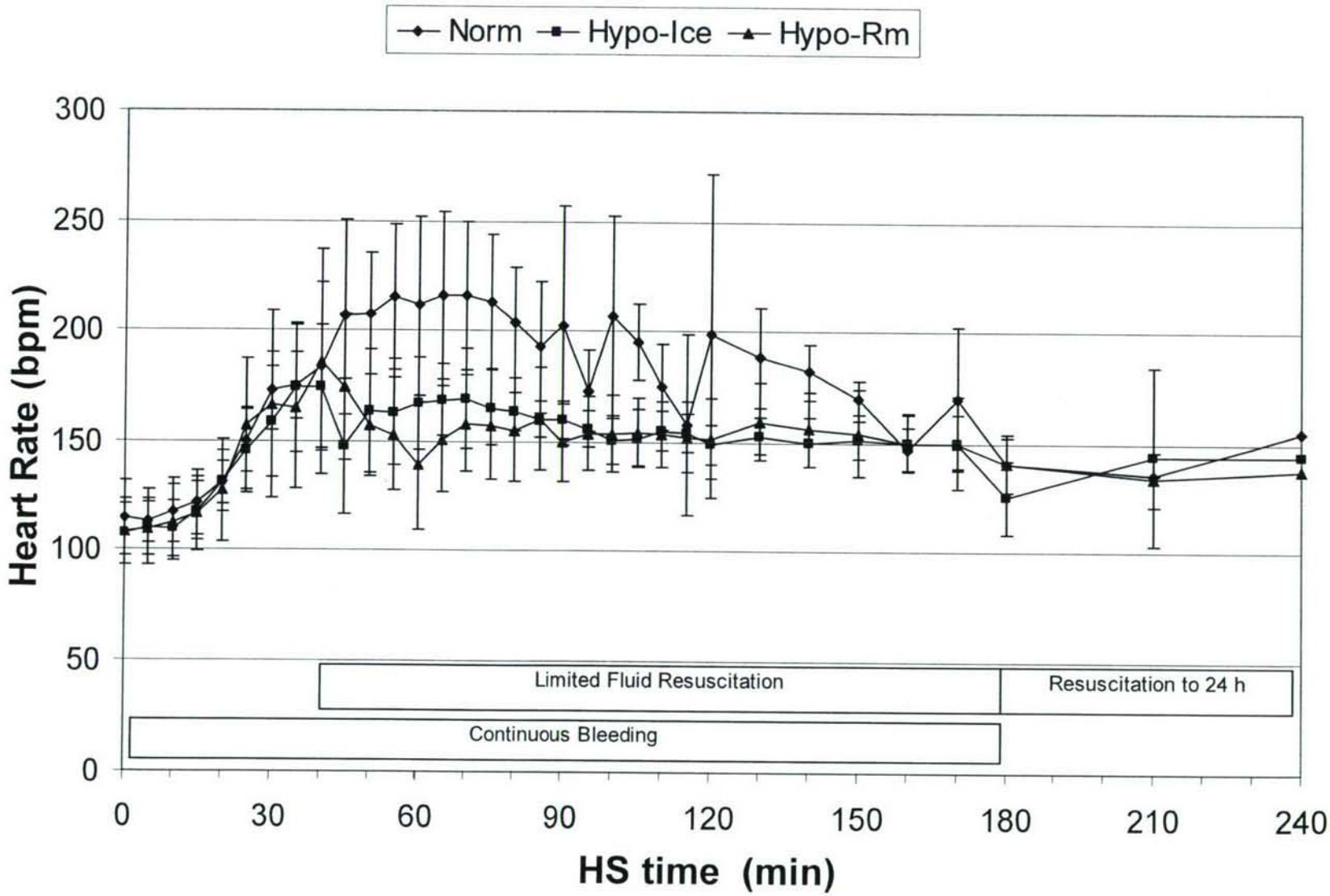

Norm
Hypo-Ice
Hypo-Rm
Heart Rate (bpm)
300
250
200
150
100
50
0
Limited Fluid Resuscitation
Resuscitation to 24 h
Continuous Bleeding
0
30
60
90
120
150
180
210
240
HS time (min)

Figure 4

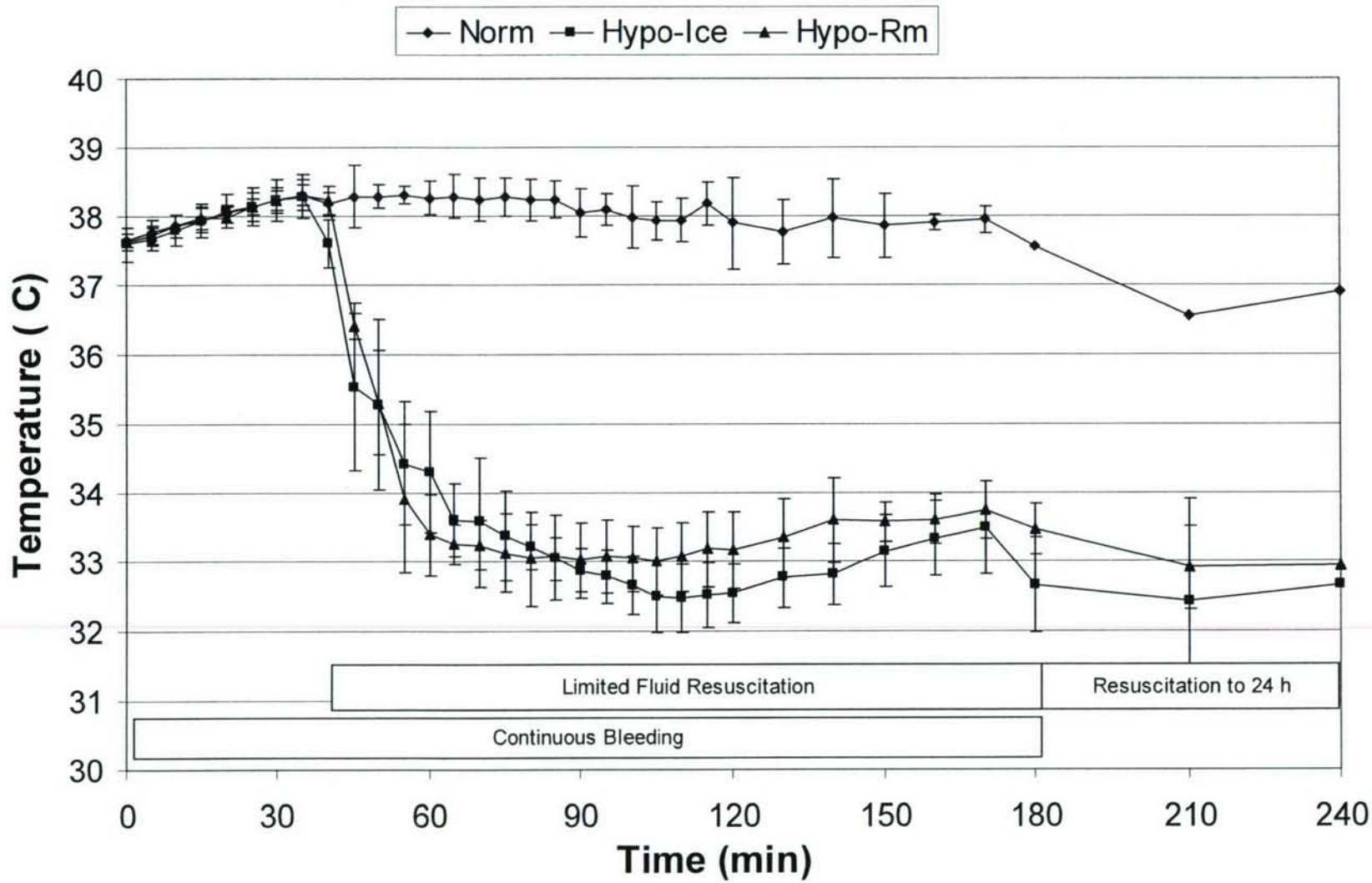
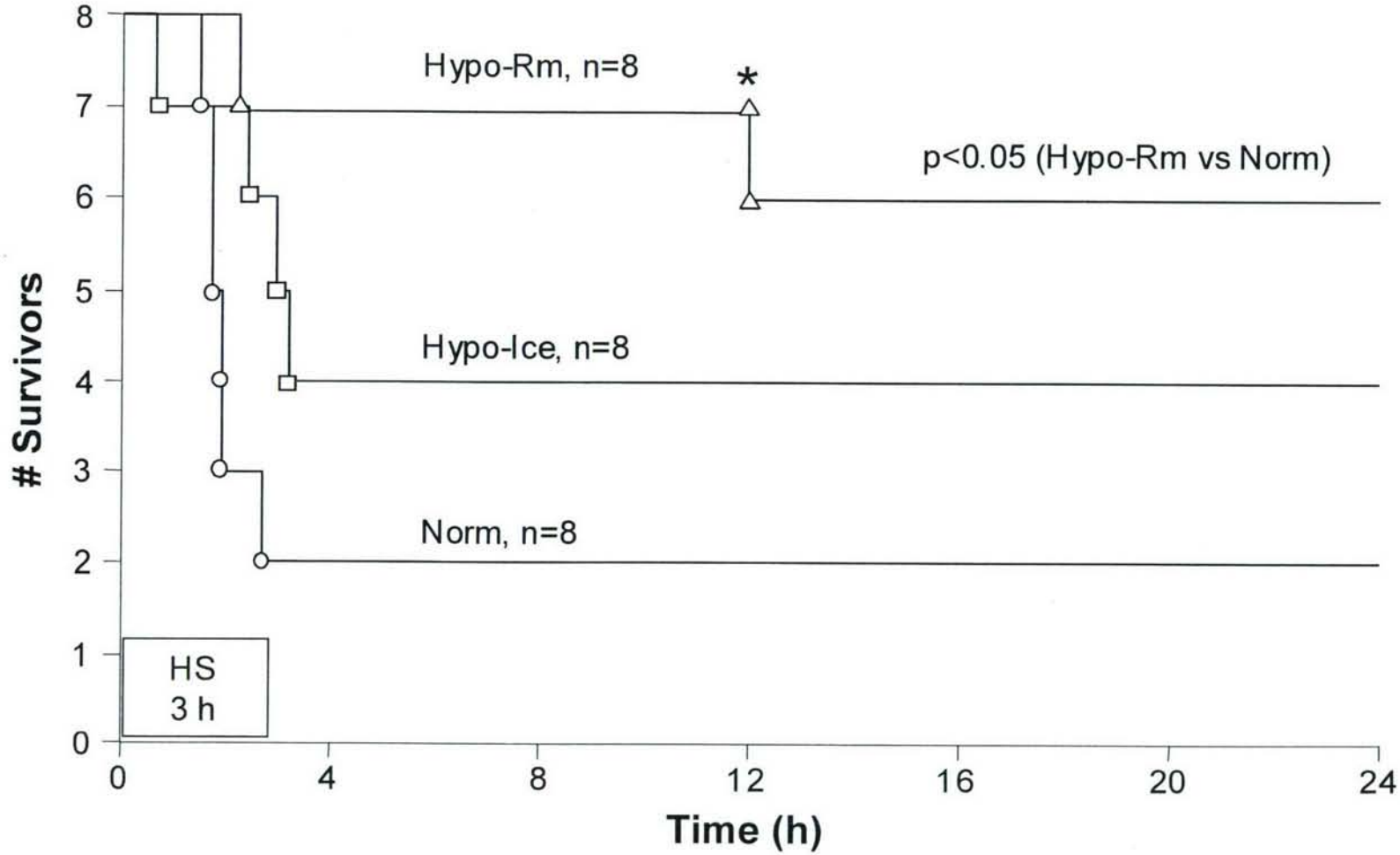

Norm
Hypo-Ice
Hypo-Rm
Temperature (C)
40
39
38
37
36
35
34
33
32
31
30
Limited Fluid Resuscitation
Resuscitation to 24 h
Continuous Bleeding
0
30
60
90
120
150
180
210
240
Time (min)

Figure 5


Hypo-Rm, n=8
*
p<0.05 (Hypo-Rm vs Norm)
Hypo-Ice, n=8
Norm, n=8
HS
3 h
Survivors
Time (h)
0
1
2
3
4
5
6
7
8
0
4
8
12
16
20
24

ACCEPTED FOR PRESENTATION AT AMERICAN ASSOCIATION
FOR THE SURGERY OF TRAUMA MEETING 2005

Session 4

Paper 37 8:40 am

MILD HYPOTHERMIA IMPROVES SURVIVAL AFTER PROLONGED, TRAUMATIC HEMORRHAGIC SHOCK IN PIGS

Xianren Wu, MD, Patrick Kochanek, MD, S. William Stezoski, Samuel A. Tisherman, MD*
Safar Center for Resuscitation Research, Departments of Anesthesiology, Critical Care Medicine, and Surgery, University of Pittsburgh

Introduction: The American Heart Association recommends therapeutic hypothermia for resuscitation after cardiac arrest. Studies in rats suggest that mild hypothermia improves survival from hemorrhagic shock (HS). Yet, the effects of hypothermia during HS are unclear since clinical studies suggest detrimental effects in trauma victims. We hypothesized that hypothermia induced with intravenous cold saline would improve survival in a clinically-relevant pig model of trauma and prolonged HS.

Methods: Domestic swine were used. After laparotomy, venous blood (75 ml/kg) was continuously withdrawn over 3 h (no systemic heparin). At HS 35 min, the spleen was transected. At HS 40 min, pigs were randomized into 3 groups (n=8, each): Group-1, normothermia (38°C) with warmed saline, Group-2, hypothermia (34°C) induced with 2°C i.v. saline and surface cooling, and Group-3, hypothermia (34°C) with 24°C i.v. saline and surface cooling. Fluids were given when mean arterial pressure (MAP) was <30 mmHg. At HS 3 h, shed blood was returned and splenectomy was performed. Intensive care, including mechanical ventilation and hemodynamic monitoring, was continued to 24 h.

Results: At 24 h, there were 2 survivors in Group-1, 4 in Group-2, and 7 in Group-3 ($p<0.05$ vs Group-1, Log Rank). Time required to achieve 34°C was 17±9 min in Group-2 and 15±4 min in Group-3 (NS). Compared to Group-3, Group-2 required less saline during HS (321±122 vs 571±184 ml, $p<0.05$). Group-2 also had a transiently higher MAP and higher lactate levels ($p<0.05$).

Conclusion: Mild hypothermia improves survival in a clinically relevant model of HS and trauma. However, administration of very low temperature (2°C) resuscitation fluid may have detrimental effects, possibly due to induced vasoconstriction and resultant MAP overshoot. During HS, infusion of 24°C saline and surface cooling are safe and effective.

209

CHRONIC ADMINISTRATION OF 17-BETA ESTRADIOL IS NEUROPROTECTIVE IN A MODEL OF CARDIAC ARREST AND CARDIOPULMONARY RESUSCITATION (CPR) IN ESTROGEN-DEFICIENT FEMALE MICE

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Introduction: The impact of estrogen replacement therapy on neurological outcome in postmenopausal females after cardiac arrest (CA) and CPR is unknown. Previous data suggests neuroprotective potency of estrogen in focal cerebral ischemia. **Hypothesis:** The purpose of this study is to determine (1) if chronic 17-beta estradiol (E2) administration in estrogen deficient female mice improves neurohistopathologic outcome after CA / CPR and (2) if effects are dose dependant. **Methods:** Anesthetized ovariectomized animals received subcutaneous silastic hormone implants, according to treatment group. 59 female C57Bl/6 mice (20-25g) were randomized to the following groups: Placebo (oil, n=19), low dose E2 (E2 6 μg, n=20) and high dose E2 (E2 12 μg, n=20). One week later, animals were re-anesthetized, ventilated and ECG monitored. CA arrest was induced by iv injection of KCl. After 10 min CPR was initiated by chest compressions (~300/min), epinephrine (6.5 μg) and 100% O2 ventilation. 72 hours after CA / CPR mice were anesthetized, blood samples for serum E2 analyses were taken followed by transcardial perfusion fixation. Damage was quantified by standard pathology (H&E) in hippocampus (CA 1) and caudoputamen (CP). **Results:** Estrogen serum levels were: oil= 29±4, E2 6= 89±14 and E2 12= 142±14 pg/ml, mean±SE. Neuronal injury in rostral CP was less after chronic E2 6 (39±7% injured neurons) and E2 12 (55±4%) administration, compared to placebo (77±2%, p<0.001). Both E2 doses improved neuronal survival in the caudal CP (E2 6: 56±6%, E2 12: 53±5%), compared to placebo (73±2%, p<0.05). CA1 injury was similar in all groups. **Conclusions:** We conclude that 17-beta estradiol replacement in estrogen deficient female mice is neuroprotective after cardiac arrest / CPR. Protection is equally robust with physiological and pharmacological doses. Estradiol may have utility as a resuscitative agent in females.

210

NOVEL DEVICE IMPROVES HEMODYNAMICS AND VITAL ORGAN PERFUSION PRESSURES DURING HYPOVOLEMIC CARDIAC ARREST IN PIGS

Demetris Yannopoulos, Cardiology, University of Minnesota, Roseville, MN; Scott McKnite, Keith G Lurie, HCMC, Minneapolis, MN

Introduction: Cardiac arrest following hypovolemic shock is associated with very poor outcomes. Cardiac output generated by CPR in this setting is limited by minimal venous return. **Hypothesis:** We tested the hypothesis that treatment of cardiac arrest after severe hypovolemia with CPR combined with a novel device (ND) that provides continuous negative intrathoracic pressure interrupted by positive pressure ventilation will improve venous return, vital organ perfusion pressures and increase mean arterial pressure without compromising oxygenation **Methods:** Six pigs were hemorrhaged to 50% of their blood volume at a rate of 60ml/min followed by 4 min of untreated ventricular fibrillation. CPR was then performed at a compression to ventilation ratio of 15:2. Each animal was treated sequentially with standard CPR (CPR) alone for 2 min and then in combination with the ND for 2 min, in an alternating fashion, for a total of 8 minutes. Aortic, right atrial, intracranial, intratracheal pressures and end tidal CO2 and O2 saturation were measured. Coronary perfusion pressure (CPP) was calculated as diastolic (aortic− right atrial pressure), cerebral perfusion pressure (CerPP) as mean aortic pressure minus mean intracranial pressure. Data from the CPR alone and CPR+ND interventions were averaged. Statistical analysis was performed by paired t-test. **Results:** Values (mean ± SEM) during CPR alone vs CPR+ND were as follows: CPP, 11.5±2.5 vs 20±2.7, p=0.008; CerPP, 6.9±3.7 vs 12.8±3.5, p=0.002; mean arterial pressure 21.3±4 vs 25.7±5, p=0.008; diastolic right atrial pressure -2.7±1.4 vs -8.2± 2.3, p=0.006; end tidal CO2 13±0.5 vs 16.3±0.9, p=0.04; and mean intratracheal pressure: 0±0 vs -9±1 p<0.001. Oxygen saturation was >99% throughout the 8 minutes of CPR in all pigs. **Conclusions:** The combination of a novel device to provide continuous negative intrathoracic pressure together with standard CPR significantly increased cerebral and coronary perfusion pressures and end tidal carbon dioxide in a hypovolemic pig model of cardiac arrest.

211

ESTABLISHMENT OF A RAT MODEL OF SUSPENDED ANIMATION WITH DELAYED RESUSCITATION: A PRELIMINARY REPORT

Tomas Drabek, Jason Stezoski, Xianren Wu, Samuel A Tisherman, S. W Stezoski, Kristin Cochran, Peter Safar, Larry Jenkins, Patrick M Kochanek, Safar Center for Resuscitation Research, University of Pittsburgh, Pittsburgh, PA

Introduction: Suspended animation (SA) is a novel approach for resuscitation of exsanguination cardiac arrest (ExCA) victims. SA utilizes cold aortic flush to induce a deep hypothermic circulatory arrest (DHCA), followed by resuscitation with cardiopulmonary bypass (CPB). In prior studies we used a dog model to maximize clinical relevance. Because of the lack of molecular tools for use in dogs, development of a rat SA model would enable study of the molecular mechanisms of neuronal injury. **Hypothesis:** We tested two hypotheses: 1) SA would be achievable in a rat model, 2) Plasmalyte (P) would be a more favorable flush solution than normal saline (NS). **Methods:** Hemorrhagic shock was induced with rapid exsanguination (12.5 ml) over 5 min, followed by KCl-induced CA. After 2 min of no-flow, cooling was initiated with ice-cold flush and surface cooling. After 30 min of DHCA, reperfusion and re-warming were achieved via CPB over 60 min. Rats were extubated 2 h later. Survival and overall performance category (OPC) were assessed at 24 h. **Results:** 18 rats were used; 4 rats died from technical reasons. Flush with ice-cold NS or P decreased tympanic temperature to 9.7±2.1 vs 11.2±2.7°C in NS vs P group. pH was 6.88±0.07 vs 6.87±0.03 in NS and P group at 5 min reperfusion (p=0.55). BE was -19±2.9 vs -21.4±2.6 in NS and P groups (p=0.13). In P group, lactate, Na and Cl were lower; glucose, K, and Mg were higher (all p≦0.05). 4/7 rats survived to 24 h in both NS and P groups. Favorable outcome (OPC1) was achieved in 2/7 rats in NS and 4/7 in P group, respectively. Pulmonary complications were seen only in NS group. **Conclusions:** We have established a SA model in rats that includes 30 min of DHCA and resuscitation using miniaturized CPB. Our data suggest a more favorable outcome with P vs NS. Successful establishment of this technically demanding model should facilitate application of molecular tools to study effects of DHCA and reperfusion on neuronal death, with relevance to cardiac surgery and transplantation medicine. Support: DAMD 17-0102-0038

212

DETRIMENTAL EFFECTS OF EPINEPHRINE ON MICROCIRCULATORY BLOOD FLOW IN A PORCINE MODEL OF CARDIAC ARREST

Michael Fries, Wanchun Tang, Yun-Te Chang, Carlos Castillo, Max H Weil, The Institute of Critical Care Medicine, Palm Springs, CA

Introduction: The use of epinephrine for the treatment of cardiac arrest is discussed controversially. We investigated the effects of epinephrine on microcirculatory blood flow in a porcine model of cardiac arrest. **Hypothesis:** Epinephrine will reduce microcirculatory flow when administered during cardiopulmonary resuscitation (CPR). **Methods:** Six pigs were subjected to five minutes of untreated ventricular fibrillation (VF) followed by five minutes of chest compression (CC) and subsequent defibrillation. Three pigs received 1 mg epinephrine after one minute of CC. Microcirculatory changes were documented using orthogonal polarization spectral imaging (OPS). Recordings were taken from sublingual tissue before onset of VF, during VF and CC and after restoration of spontaneous circulation (ROSC). Microcirculatory flow was assessed using a semiquantitative score (0=no flow, 1=sluggish, 2=reduced, 3=normal). **Results:** In pigs that were treated with epinephrine microcirculatory flow was significantly reduced when compared to untreated animals. These effects were present for at least five minutes and persisted even when ROSC was achieved. **Conclusions:** Microcirculatory flow is altered for at least five minutes after a single administration of 1 mg epinephrine during CPR and may contribute to further tissue damage especially in vulnerable organs.

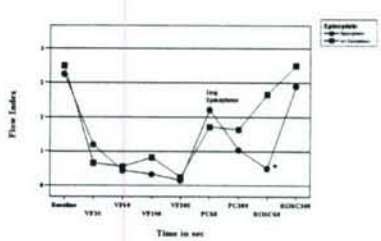

Fig. 1: Comparison of microvascular flow index in animals with and without treatment of epinephrine. *indicates significant differences between groups (p<0.05).

81

SUSPENDED ANIMATION WITH DELAYED RESUSCITATION ALLOWS INTACT SURVIVAL FROM CARDIAC ARREST RESULTING FROM PROLONGED LETHAL HEMORRHAGE IN DOGS

Xianren Wu, Tomas Drabek, Samuel A Tisherman, Jeremy Henchir, William Stezoski, Kristin Cochran, Patrick M Kochanek, Safar Center for Resuscitation Research, Univ. of Pittsburgh, Pittsburgh, PA; Robert Garman, Veterinary Pathology, Inc., Murrysville, PA

Introduction: Most combat fatalities result from rapid exsanguination. Conventional resuscitation is often unsuccessful. Previously, we reported the success of a novel approach called suspended animation (SA) with delayed resuscitation in exsanguination cardiac arrest (ExCA). SA of up to 2 h was induced via rapid aortic flush with ice-cold (10°C) saline followed by delayed resuscitation via cardiopulmonary bypass (CPB). This would buy time for transport and surgical repair. We used SA to achieve intact survival of dogs after rapid hemorrhage (over 5 min) to ExCA. **Hypothesis:** SA will allow survival with good neurological outcome in the setting of prolonged hemorrhage prior to ExCA. **Methods:** Dogs underwent controlled, continuous bleeding until CA. Two min after CA, dogs were randomized into 3 groups (n=7 each): 1) CPR Group resuscitated with conventional CPR and rapid infusion of blood and LR; 2) SA-1, or 3)SA-2 Groups, both of which received 20 L of 2°C saline flushed into the aorta. CPR or SA lasted 60 min, and was followed by 2h of CPB. CPR dogs were maintained at 38.0°C, while SA dogs were controlled at 34 °C for either 12h (SA-1) or 36h (SA-2). Outcome was evaluated with Neurological Deficit Scores (NDS) (0%=normal, 100%=brain death) and Overall Performance Category (OPC) (1=normal, 5=death). **Results:** CA occurred after 124±16 min of hemorrhage. In the CPR group, spontaneous circulation could not be restored without CPB; none achieved long-term survival (range: 11.5-16.5 h). Twelve of 14 SA dogs survived ($p<0.01$ vs CPR group). SA-2 group had lower NDS than SA-1 [1.5 (0-89%) vs 42 (10-92%)] ($p=0.04$) and better OPC (5 vs 1 dog recovered to normal)($p=0.06$). **Conclusions:** SA facilitated survival with good neurological outcome in a model of otherwise unresuscitable prolonged hemorrhage with ExCA. Surprisingly, extending the duration of mild hypothermia after SA was critical to achieving intact neurologic outcome. Supported by USAMRMC DAMD 17-01-2-0038

82

POLY-ADP-RIBOSYLATION AS A POST-TRANSLATIONAL MODIFICATION OF MITOCHONDRIAL PROTEINS

Yi-Chen Lai, Y. Chen, X. Zhang, P.M. Kochanek, P.D. Nathaniel, L. Jenkins, R.S.B. Clark, Safar Center, U. of Pittsburgh, Pittsburgh, PA; C. Szabo, Inoteck Corp, Beverly, MA

Introduction: Poly-ADP-ribosylation (PAR) is an important post-translational modification facilitated by poly(ADP-ribose) polymerase (PARP). PARP activation is important in many homeostatic processes including DNA repair, transcriptional regulation, and memory; however, PARP overactivation contributes to energy failure (ATP and NAD depletion) and cell death via apoptogenic proteins from the mitochondria. After experimental traumatic brain injury (TBI), PARP also appears to have both detrimental and beneficial roles. **Hypothesis:** PARP regulates mitochondrial processes involved in energetics and apoptosis. **Methods:** A targeted proteomics approach was used to identify PARP substrates. Whole brain mitochondria from adult rats were isolated and tested during nitrosative stress. Viability was verified by O2 consumption. Structural integrity and the presence of both PARP and PAR were verified by immuno-electronmicroscopy. Mitochondrial proteins were also obtained from injured cerebral cortex 24 h after TBI or from naive control rats (n=4/group). Mitochondrial proteins were isolated and immunoprecipitated using an antibody against poly(ADP-ribose). Immunoprecipitates were separated on 2-D gels and selected peptides were analyzed by matrix assisted laser desorption/ionization mass spectroscopy. Identified proteins of interest were verified by Western Blot. **Results:** PAR of several (>50) mitochondrial was observed. Overall, PAR of proteins was reduced in both isolated mitochondria after nitrosative stress and mitochondria after TBI in vivo. Several potentially important proteins were identified as PARP substrates, including: β subunit of F1F0 ATPase (complex V), voltage-dependent anion channel (a key component of the mitochondrial membrane permeability transition pore [MPTP]), actin, tubulin, creatine kinase, and 14-3-3. **Conclusions:** These data suggest that PAR is a prevalent post-translational modification in mitochondria, and that PARP may regulate ATP synthesis and the release of apoptogenic factors from the mitochondria via the MPTP. Support NS38620/NS30318/HD40686

83

MITOCHONDRIAL DYSFUNCTION AFTER TRAUMATIC BRAIN INJURY IN IMMATURE RATS

Courtney L. Robertson, Manda Saraswati, Pediatrics, University of Maryland, Baltimore, MD; Gary Fiskum, Anesthesiology, University of Maryland, Baltimore, MD

Introduction: Although traumatic brain injury (TBI) is a leading cause of death in children, the mechanisms responsible for neuronal death in the developing brain are unknown. Evidence suggests that mitochondria are important mediators of cell death in models of TBI in adult rats. **Hypothesis:** We hypothesized that TBI would lead to mitochondrial dysfunction early after injury in a rat model of pediatric TBI. **Methods:** Immature rats (PND 17) underwent controlled cortical impact (CCI, n=8) or sham injury (n=6) to the left temporal cortex. One hour after CCI, mitochondria were isolated separately from both the ipsilateral (left) and contralateral hemispheres, and evaluated for repiration, reactive oxygen species (ROS) generation and cytochrome c content. Rates of phosphorylating (State 3) and resting (State 4) respiration were measured with and without bovine serum albumin (BSA). The acceptor control ratio (ACR) was calculated (State 3/State 4) as an indicator of mitochondria functional integrity. **Results:** Mitochondrial State 4 rates were higher in trauma versus control or sham (State 4 left/right=1.4 for trauma vs 0.9 for sham, $p<0.05$). State 3 rates were similar between sides, with a reduced ACR in trauma mitochondria (7) versus contralateral (9.0) or sham (9.5). BSA reversed the elevation of State 4 after CCI, with a mean trauma ACR the same as contralateral and sham. Mitochondrial ROS generation did not differ between hemispheres. Mitochondrial pellets from injured animals had reduced cytochrome c content compared to sham animals, with the greatest reduction seen on the injured side. **Conclusions:** These data demonstrate that mitochondrial dysfunction occurs early after TBI in the developing brain. The reversibility with BSA suggests that free fatty acids present after TBI may lead to mitochondrial membrane alterations. Future studies should correlate mitochondrial findings with histologic and neurologic outcome. This could identify novel targets for neuroprotection after TBI in infants and children. Support: K08NS42805

84

AGE-RELATED AND GENDER-BASED DIFFERENCES IN GENE EXPRESSION OF BLOOD BRAIN BARRIER (BBB) NUTRIENT TRANSPORTERS

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Introduction: Delivery of glucose and monocarboxylic acids across the microvessels (mv) of the BBB requires specific transporter proteins, GLUT1 and MCT1, respectively. Previous studies support a post-natal increase in BBB GLUT1 mRNA and protein in association with a comparable decrease in MCT1. Mechanisms for coordinated regulation of these genes are unknown. **Hypothesis:** We designed a study to generate pure fractions of BBB RNA for measurement of gene expression and to investigate age-related and gender-based differences in gene expression. **Methods:** BBB mv were prepared from whole forebrains from post-natal (P)15 and P28 day old rats (6-8 rats each sex). Mv were isolated in 17% dextran and final filtration through 40 μm mesh. Gene expression was determined by quantitative RT-PCR. cDNA was synthesized using random hexamers from total RNA, which was extracted using TRIZOL reagent. Data were normalized for each run and triplicate experiments were performed. GLUT1 protein expression was determined by Western blot. **Results:** 5.2 ± 3.4 (Mean ± SD) fold increase in GLUT1 expression was demonstrated with a concomitant 4.0 ± 2.5 fold decrease in MCT1 expression with development (P28 vs. P15 rats). 2.2 ± 0.6 and 1.3 ± 0.9 fold increase in GLUT1 and MCT1 expression, respectively was seen in P28 female rats as compared with their male counterparts. However, GLUT1 greater protein was seen in P28 male rats. **Conclusions:** This is the first investigation to measure gene expression in isolated BBB mv. The data confirm a maturational change in encoded RNA for GLUT1 and MCT1 and suggest possible transcriptional control. This is also the first investigation of gender-based differences in expression of BBB nutrient transporters. The difference in females of GLUT1 and MCT1 mRNA suggests a possible role of sex hormones in the control of such expression. The concomitant greater expression of GLUT1 protein in P28 male rats suggests separate translational control. Finally, this study establishes a system for study of genetic regulation of the mammalian BBB. Supported by HD 30704.

122

ECHOCARDIOGRAPHY TO ASSESS POSTBURN MYOCARDIAL FUNCTION

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Introduction: Echocardiography provides non-invasive and clinically relevant assessment of LV function in injury and disease. We determined if transthoracic echocardiography (Echo) in awake mice correlated with *in vitro* assessment of LV function using isolated hearts (Langendorff). **Methods:** Burn over 40% TBSA (or sham burn) was given in C57 BL6 mice 22-24 g; lactated Ringers was given IP. Echo was performed at baseline (BL) and at designated intervals over 7 days postburn (Table, mean ±SEM. *indicates difference from BL at p<0.05, ANOVA Student Neuman Keuls). Subgroups of mice were sacrificed at intervals and hearts perfused to assess LVP and ±dP/dt responses to ionotropic challenge (N=8/group/time). **Results:** Burn produced myocardial depression evidence by fall in ejection fraction 12, 24, 48, 72 hrs postburn and decreased LVP and ±dP/dt max. Myocardial recovery occurred by Day 7 postburn. **Conclusions:** Echo provides measures of *in vivo* cardiac function which correlate with contractile depression measured using *in vitro* Langendorff. Echo is a valuable tool for non-invasive assessment of postburn myocardial function. **Supported by NIH 2P50 GM21681-39.**

% EJECTION FRACTION (IN VIVO)						
	BASELINE	12 HR	24 HR	48 HR	72 HR	7 DAY
SHAM	67±2	67±2	69±2	69±1	69±2	70±1
BURN	68±2	50±3*	58±3*	61±2*	63±1*	72±1
LEFT VENTRICULAR PRESSURE (IN VITRO)						
SHAM	96±2	96±2	97±1	96±1	98±1	99±3
BURN	96±1	84±7	63±1*	80±4*	93±7	102±2

123

RECTAL PCO2 MONITORING: A COMPARISON OF CONTINUOUS MONITORING METHODS

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Introduction: Hypoxia, a major problem for critically ill patients, has been linked to the development of organ failure. SL-capnometery reliably measures PCO2, a marker of gut hypoperfusion. While SL-PCO2 monitoring avoids the major problems surrounding gastric measurement, other factors may interfere with accurate SL-PCO2 measurement (hyper/hypoventilation, loss of contact with SL-mucosa). Thus, measurement at another site,the rectum, may effectively limit error. **Hypothesis:** Continuous PCO2-monitoring in the rectum using a sublingual-CO2 System (RSL-PCO2) and microelectrode technology (RM-PCO2) during progressive hemorrhage in a rat model will yield similar values. **Methods:** Anesthetized Wistar rats were progressively hemorrhaged/reperfused. A modified SL-Probe and microelectrode were inserted into the rectum. Data were collected per minute. **Results:** Data from 10 control and 10 experimental rats are reported as mean+SEM. The model produced changes in RSL-PCO2 and RM-PCO2 between the control and experimental condition (p<.05). Control PCO2 exceeded experimental PCO2 at baseline, especially the RM-PCO2. Significant differences in RM-PCO2 occurred between the control and experimental group at 60 and 120 min. hemorrhage. RSL-PCO2 values approached significance between the control and experimental group at 60 (p=0.08) and 120 min. (p=0.05). A moderate correlation between the two measures occurred for the control group (r=.59), where RSL-PCO2 averaged 3.0 torr (+0.4 torr) higher than RM-PCO2. A higher correlation was noted under experimental conditions (r=.81) where the difference between methods was reduced to 0.64 torr (+0.25 torr). Agreement, calculated using the Bland-Altman technique, revealed the 90% limits of agreement was 3.0+19.7 torr for the controls and 0.6+16.1 torr for the experimental group. **Conclusions:** While mean arterial pressure stabilized, baseline PCO2 for both measures apparently did not reach equilibrium. Tracking during hemorrhage was similar using both methods. Accurate monitoring of rectal-PCO2 may be a useful adjunct for triage and to monitor and guide the management of trauma patients.

124

NEUROTENSIN FAILS TO AUGMENT THE EFFECT OF INDUCED MILD HYPOTHERMIA ON SURVIVAL AFTER HEMORRHAGIC SHOCK IN AWAKE RATS

Xianren Wu, Jason Stezoski, Patrick M Kochanek, Samuel A Tisherman, Safar Center for Resuscitation Research, Univ. of Pittsburgh, Pittsburgh, PA; Elliott Richelson, Mayo Clinic, Jacksonville, FL; Laurence Katz, Dept. of Emerg Med, Chapel Hill, NC

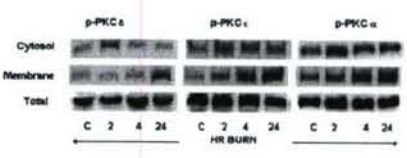
Introduction: We reported that mild hypothermia (HTH, either induced or spontaneous) improves survival after hemorrhagic shock (HS) in rats. Administration of the neurotensin analog (NT69L), a potent poikilothermic agent, facilitated the beneficial effects of hypothermia in cardiac arrest in rats, perhaps by minimizing cold-induced stress. In contrast, NT69L did not alter either the temperature course of spontaneous HTH or survival after HS in rats. **Hypothesis:** Administration of NT69L would enhance the impact of induced HTH on survival during HS in awake rats. **Methods:** Sprague-Dawley rats were prepared under isofluorane anesthesia. After instrumentation, rats were placed prone in a restrainer and 30 min were allowed for emergence from anesthesia. Venous blood 42.5 ml/kg was withdrawn over 35 min. Starting from HS 40 min, 6% Hetastarch was infused i.v. if mean arterial pressure (MAP) decreased below 50 mmHg. Rats were randomized at HS 40 min into 3 groups (n=10, each): Group 1, spontaneous HTH, Group 2 and 3, induced HTH using surface cooling from HS 45 min to cool to rectal temperature (Tr) 34°C. Group 3 received NT69L (0.5 mg/kg) during HS 40-50 min, while Group 1 and 2 received vehicle. Shed blood and lactated Ringer's were given after HS 210 min. Tr was maintained at the target level for 12 h. Observation was continued to 72 h. **Results:** At HS 210 min, the Tr was 34.4 ±0.5°C in Group 1. The mean time to reach Tr 34°C was 15 min in group 2 and 20 min in group 3 (p<0.05 vs Group 1). There was no significant difference between groups in MAP, heart rate and physiologic parameters. The time to decompensation (MAP<50 mmHg) was not different between groups. By 72h, there were 2 survivors in each group and the survival times were not different between groups. **Conclusions:** Rapidly induced mild HTH does not improve survival in our awake HS model in rats compared to spontaneous HTH. Administration of NT69L does not modify the effects of induced HTH during HS.

125

ROLE OF PKC ISOZYMES IN BURN-RELATED CONTRACTILE DYSFUNCTION

Jing Tan, D. Jean White, David L Maass, Jureta W Horton, Surgery, UT Southwestern Medical Center at Dallas, Dallas, TX

Introduction: Protein kinase C (PKC) is a family of isozymes that regulates many aspects of cell/organ function and has been implicated in the myocardial dysfunction that occurs with injury such as burn trauma. This present study examined burn-related alterations in myocardial PKCε, α, δ to determine the specific PKC isozymes involved in myocardial responses to major burn. **Methods:** SD rats, 350g were given burn over 40% TBSA (or sham burn for controls) and fluid resuscitated, lactated Ringers, 4ml/kg/% burn. Subgroups of burns and shams were sacrificed either 2, 4, or 24 hrs postburn (N=5 rats/time/group); total/phosphorylated PKC isozymes were measured in myocardial membrane and cytosol fractions. Additional subgroups of rats (N=8 rats/time/group) were used to examine myocardial function (Langendorff). **Results:** Burn injury produced translocation of PKC from cytosol to myocardial membrane fraction and a significant rise in phosphorylated membrane PKCε and αwhile the other isozyme was not significantly changed. Burn-related alterations in PKCε and α preceded myocardial contraction/relaxation defects. **Conclusions:** Burn injury produces myocardial PKC activation, likely contributing to postburn myocardial contractile depression. Supported by **NIH R01 GM57054-06.**



359

ACTIVATION OF THE INTRINSIC AND EXTRINSIC CASPASE PATHWAYS FOLLOWING EXPERIMENTAL INTRAUTERINE INFLAMMATION

Aditi Sharangpani, Michael J Bell, Critical Care Medicine, Children's National Medical Center, Washington, DC

Introduction: Inflammation during pregnancy has been implicated in developmental diseases of the white matter, such as cerebral palsy. Our model of inflammation results in increased apoptosis in fetal brains. Caspase-mediated apoptosis can occur by activation of the intrinsic (through actions of caspase-9 (c-9)) or the extrinsic (through actions of caspase-8 (c-8) or caspase-10 (c-10)) pathways. Both of these pathways lead to activation of the effector caspase-3 (c-3), ultimately resulting in cell death. **Hypothesis:** We hypothesized that c-8, c-9 and c-10 activation would precede activation of c-3. **Methods:** Pregnant rats were injected (lipopolysaccharide, 0.1 mg/kg) intracervically at E15, fetuses harvested at baseline (base), 1h, 2h, 4h and 16h (n = 22, 6, 11, 11 and 13, respectively), and brains homogenized. Caspase activities were measured fluorometrically, normalized for protein concentration, and compared using One Way ANOVA. **Results:** Since apoptosis occurs throughout brain development, all data are presented as changes from base. C-9 activity increased at 1h (+140%, $p < 0.05$) and 2h (+70%, $p < 0.05$). C-10 increased at 1h (+46%, $p < 0.05$) and 2h (+8%, $p < 0.05$) with a decrease from base at 16h (-72%, $p < 0.05$). C-3 activity did not vary from base during the study period. C-8 activity decreased from base at 16h (-40%, $p < 0.05$) but was otherwise unchanged. **Conclusions:** Caspases involved in the intrinsic and extrinsic pathways are activated within hours of our inflammatory stimulus. We have previously shown that expression of Fas, a cell surface receptor involved in initiation of the extrinsic caspase pathway, is increased within hours in response to our stimulus. This is our first evidence of activation of the intrinsic pathway, implying that cellular stress may also play a role in cell death in our model. We did not observe an effector caspase response. It is possible that this response occurs beyond the time points of our experiment.

360

RAT HIPPOCAMPAL DEGRADOMICS DURING 30 MINUTES OF GLOBAL CEREBRAL ISCHEMIA WITH & WITHOUT HYPOTHERMIA TREATMENT

Mandeep S Chadha, Patrick M Kochanek, Critical Care Medicine, U. of Pittsburgh, Pittsburgh, PA; Larry W Jenkins, Neurological Surgery, U. of Pittsburgh, Pittsburgh, PA; Thomas Drabek, Jason Stezoski, Safar Center for Resuscitation Research, U. of Pittsburgh, Pittsburgh, PA

Introduction: Degradomics is the study of global protein degradation during injury or disease. Prior studies from our laboratory using a large format 2D gel proteomic approach showed surprisingly minimal differences in protein degradation when comparing 30 min of global cerebral ischemia (GCI) at either 38 or 10C. However, weaker solublization buffers are required for 2D vs 1D gel analysis. We sought to further evaluate global protein degradation during GCI with and without hypothermia using more powerful solublization buffers and 1D gel analysis. **Hypothesis:** Protein degradation is minimal during prolonged normothermic or hypothermic ischemia in rat brain. **Methods:** Using a decapitation complete ischemia model in Sprague-Dawley rats (n=6/group), both hippocampi were rapidly dissected and randomized to 30 min of complete ischemia at either 38 or 10C. A third group of hippocampi (no ischemia) served as controls. Separation of proteins from hippocampal lysates by molecular weight was accomplished with medium format (16x18 cm) SDS-PAGE. Paired samples were run in triplicate on the same gel to reduce variability, stained with Sypro Ruby, imaged, and quantified. **Results:** No differences in protein levels were found between either normothermic or hypothermic ischemia groups or controls without reperfusion (one-sample sign test). **Conclusions:** We observed little degradation of the rat hippocampal proteome during prolonged normothermic and hypothermic complete ischemia without reperfusion. These data confirm and extend our prior work with 2D gel proteomics. In that multiple studies have shown important neuroprotection by profound hypothermia during GCI, we are currently examining the effect of reperfusion on hippocampal protein degradation after prolonged normothermic and deep hypothermic circulatory arrest in rats using miniaturized cardiopulmonary bypass. Support: DOD DAMD17-01-2-0038, NS 35365, T-32 HD 40686.

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Title: Suspended Animation With Delayed Resuscitation Allows Intact Survival From Cardiac Arrest Resulting From Prolonged Lethal Hemorrhage In Dogs

Introduction: Suspended Animation (SA) is a novel concept for otherwise hopeless cardiac arrest (CA) from trauma and hemorrhage. Previously, we achieved intact survival of dogs from up to 2 h of CA resulting from rapid hemorrhage (over 5 min). Now we tested if SA would allow survival with good neurological outcome in the setting of prolonged hemorrhage prior to CA.

Methods: Two min after CA resulting from prolonged hemorrhage, dogs were randomized into 3 groups (n=7 each): 1) CPR Group resuscitated with conventional CPR and rapid infusion of blood and lactated Ringer's; 2) SA-1, or 3) SA-2 Groups, both of which received 20 L of 2°C saline flushed into the aorta. CPR or SA lasted 60 min, and was followed by 2 h of resuscitation by cardiopulmonary bypass. CPR dogs were maintained at 38.0°C, while SA dogs were controlled at 34°C for either 12 h (SA-1) or 36 h (SA-2). Functional outcome and brain histology were evaluated at 72 (SA-1) or 96 h (SA-2).

Results: CA occurred after 124±16 min of hemorrhage. In the CPR group, spontaneous circulation could not be restored without CPB; none achieved long-term survival. In contrast, 12 of 14 SA dogs survived. Compared to SA-1, SA-2 had lower Neurological Deficit Scores ($p=0.04$), and lower Histologic Deficit Scores in the neocortex ($p<0.05$).

Conclusions: SA facilitated survival and neurological recovery in a model of otherwise unresuscitable CA resulting from prolonged hemorrhage. Extended mild hypothermia during ICU recovery after SA was critical to achieving intact neurological outcome and may have implications in the setting of deep hypothermia CA.

The author affirms that the abstract herein will not have been published as a manuscript prior to presentation at the American Heart Association meeting, that any animal studies conform with the "Position of the American Heart Association on Research Animal Use" (Circulation 1985; 71:849), and that any human experimentation has been conducted according to a protocol approved by the institutional committee on ethics of human investigation or, if no such committee exists, that it conforms with the principles of the Declaration of Helsinki of the World Medical Association (Clinical Research 1966; 14:103).

The Submitting author also certifies that all authors named in this abstract have agreed to its submission for presentation at the AHA Fellow's Research Day and are familiar with the 10-author rule ("Rules for Submission of Abstracts").

Submitting author's signature

P4

SUSPENDED ANIMATION ALLOWS NORMAL RECOVERY AFTER EXSANGUINATION CARDIAC ARREST IN RATS.

T. Drabek, J. Stezoski, L. Jenkins, M. Chadha, X. Wu, W. Stezoski, J. Henchir, S. Tisherman, P. Kochanek (Support: DAMD 17-0102-0038) Safar Center for Resuscitation Research, University of Pittsburgh School of Medicine, Pittsburgh, PA 15260

Introduction: Suspended animation (SA) is a novel method for resuscitation of exsanguination cardiac arrest (ExCA) using aortic flush to achieve deep hypothermia circulatory arrest (DHCA) followed by delayed resuscitation. We have successfully studied SA in a dog model. A rat SA model would enable study of mechanisms and drug screening.

Hypothesis: Survival from 20 min SA at 15°C after ExCA is achievable.

Methods: ExCA was achieved by removal of 12.5 ml of blood over 5 min, followed by KCl-induced CA and 1 min of no-flow. Three groups were studied: (1) hypothermic SA (H-SA, 0°C flush with Plasma-Lyte A, n=5); (2) normothermic SA (N-SA, 38°C flush, n=6); (3) control group (n=6). After 20 min of H-SA or N-SA, resuscitation was attempted via miniaturized CPB over 60 min. Controls were subjected to 60 min CPB only. Surviving rats were weaned from mechanical ventilation and extubated 2 h later. Survival, Overall Performance Category (OPC), Neurologic Deficit Score (NDS) and weight were assessed at Day 7.

Results: All rats in H-SA and control groups achieved OPC 1. None of the rats in N-SA group had restored cardiac activity or survived. NDS was normal in H-SA and control rats. There were no differences in NDS (2±4.5 vs. 7.5±8.8; p=0.24) and weight (339±9 g vs. 339±29 g; p=0.96) between H-SA and control groups.

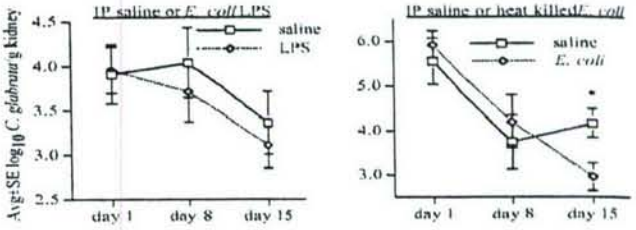
Conclusion: We have established a rat SA model that includes 20 min of SA. This new model should greatly facilitate study of both mechanisms of neuronal injury and therapies in DHCA.

P5

EFFECT OF LPS AND NONVIABLE *ESCHERICHIA COLI* ON MURINE *CANDIDA GLABRATA* CANDIDEMIA.

R. Garni, M.A. Johnson*, C. Bendel*, M. Henry-Stanley and C. Wells. Univ. of MN, Minneapolis, 55455, USA.

Candida glabrata is now the 2nd or 3rd most frequent cause of candidemia (after *C. albicans*) in trauma patients. Although considered less pathogenic than *C. albicans*, *C. glabrata* candidemia has similarly high mortality (~30-40%). Relatively little is known about *C. glabrata* pathogenesis, but patients with a *C. glabrata* blood isolate often have concomitant or preceding isolation of a bacterium or another *Candida* sp. Because others have reported that *C. albicans* candidemia can be augmented by intact *E. coli* or by *E. coli* LPS, experiments were designed to clarify the effect of *E. coli*, and *E. coli* LPS, on *C. glabrata* candidemia. Mice were inoculated IV with 10^8 *C. glabrata* and sacrificed 1, 8 and 15 days later. To study the effect of LPS on the course of systemic *C. glabrata* infection, mice were also inoculated 16 hr before each sacrifice with IP saline or with IP 100 μg *E. coli* LPS (n=10-15/treatment/ day). Compared to saline controls, fewer *C. glabrata* were recovered from the kidneys (left figure) and livers (not shown) of LPS-treated mice, although these differences were not significant. In a follow-up experiment, $10^{8.7\text{-}9.7}$ heat-killed *E. coli* was substituted for LPS. In mice with long-standing (15-day) *C. glabrata* candidemia, IP *E. coli* was associated with a 14-fold ($P<0.05$) and 6-fold ($P=0.1$) decrease in the numbers of *C. glabrata* recovered from kidneys (right figure) and livers (not shown), respectively. Thus, *E. coli*



LPS and intact *E. coli* did not augment *C. glabrata* candidemia, and these bacterial agents actually appeared to ameliorate the course of systemic *C. glabrata* infection.

P6

VAGUS NERVE PREVENTS SYSTEMIC INFLAMMATION BY INHIBITING TNF TRANSCRIPTION IN THE SPLEEN.

Luis Ulloa, Hong Liao*, Mahendar Ochani*, Kanta Ochani*, Jared M. Huston*, Christopher J. Czura, & Kevin J. Tracey . Center of Immunology and Inflammation, North Shore-LIJ Research Institute. North Shore University Hospital. 350 Community Drive, Manhasset, New York 11030. Lulloa@nshs.edu

Endotoxin stimulates macrophages to release pro-inflammatory cytokines including tumor necrosis factor (TNF), which can cause lethal shock and tissue injury. We have recently discovered that vagus nerve stimulation attenuates circulating TNF levels during endotoxemia. This mechanism was named "the cholinergic anti-inflammatory pathway" because acetylcholine, the principle neurotransmitter of the vagus nerve, inhibits TNF production in macrophages. The object of this study is to determine how vagus nerve regulates TNF production in different organs. Here we report that stimulation of the vagus nerve prevents lethal systemic inflammation by inhibiting TNF transcription in the spleen. Splenectomy attenuates circulating TNF levels during endotoxemia (sham = 181.3 +/- 31 pg TNF/ mL serum vs. splenectomy = 41.2 +/- 13.4 pg TNF/mL serum; $p<0.05$). Vagus nerve stimulation fails to further attenuate circulating TNF levels in splenectomized mice. Electrical stimulation of the vagus nerve activates a splenic neuronal network, and sectioning of the common celiac trunk of the vagus nerve, proximal to the spleen, ablates vagus nerve regulation of TNF in both

SUSPENDED ANIMATION ALLOWS NORMAL RECOVERY AFTER EXSANGUINATION CARDIAC ARREST IN RATS

Tomas Drabek, M.D., 1, Jason Stezoski, M.S., Xianren Wu, M.D., Larry Jenkins, Ph.D. and Patrick M. Kochanek, M.D.
[1]Safar Center for Resuscitation Research, University of Pittsburgh School of Medicine, Pittsburgh, PA, United States.

Suspended animation (SA) or "Applied Emergency Hypothermia" is a promising technique to increase the survival of patients with extreme, rapid blood loss that often is experienced in traumatic civilian and battlefield injuries. Research into SA has been somewhat hindered due to the lack of a small animal model of SA that could be used to study what mechanisms are at work in the body that make SA possible and effective. Researchers at the University of Pittsburgh have developed a rat model that can be used for SA research, promising to expedite SA research and therapies.

In civilians, 50% of deaths due to trauma occur at the scene, and another 30% within hours from injury. A similar situation has been described in the military setting. Conventional approaches to resuscitate these victims with exsanguination cardiac arrest (ExCA), which is cardiac arrest caused by extreme blood loss, are generally unsuccessful. However, in an appropriate setting, some of those traumatic injuries could be surgically repaired, but often there is not enough time to transport the patient to the appropriate setting.

In 1984, the late Dr. Peter Safar of the University of Pittsburgh, the Father of modern CPR, and Col. Ronald Bellamy, a US Army surgeon, an expert on trauma deaths in the Vietnam conflict, developed a novel approach to the resuscitation of victims of ExCA which was called suspended animation with delayed resuscitation. Well aware of the effects of hypothermia on reducing metabolic demands and conferring additional cellular protection, they advocated to infuse an ice-cold solution to rapidly reduce the victim's body temperature after the cardiac arrest. This approach was selected to buy time to transfer the victim to a surgical facility where either damage control surgery or definitive repair could be carried out. After repair, a delayed resuscitation was proposed using cardiopulmonary bypass (CPB) -- the traditional heart-lung machine used in cardiac surgery.

For a period of over 15 years, to demonstrate the efficacy of this approach, studies were carried out in large animal models. However, these experiments were expensive, limiting the ability to screen large numbers of new adjunctive therapies to the use of hypothermia. Additionally, recent studies in molecular biology have produced a number of tools for use in rat models and that are unusable in larger animal models. The absence of an effective rat model of SA is a significant obstacle to the advancement of this life-saving research.

In the University of Pittsburgh study, anesthetized rats were hemorrhaged over 5 minutes, and a cardiac arrest was then induced for one additional minute. Then, an ice-cold solution (0°C) or a normothermic solution (37°C) was infused to lower the body temperature or serve as a control, respectively. With the cold flush, the temperature of the rat decreased to 15°C inducing the suspended animation condition. After twenty minutes of suspended animation, resuscitation with CPB was started. The control rats were subjected to the same duration of CPB only. The surviving rats were mechanically ventilated for two hours, and after weaning from ventilator put in a cage to be observed for next 7 days.

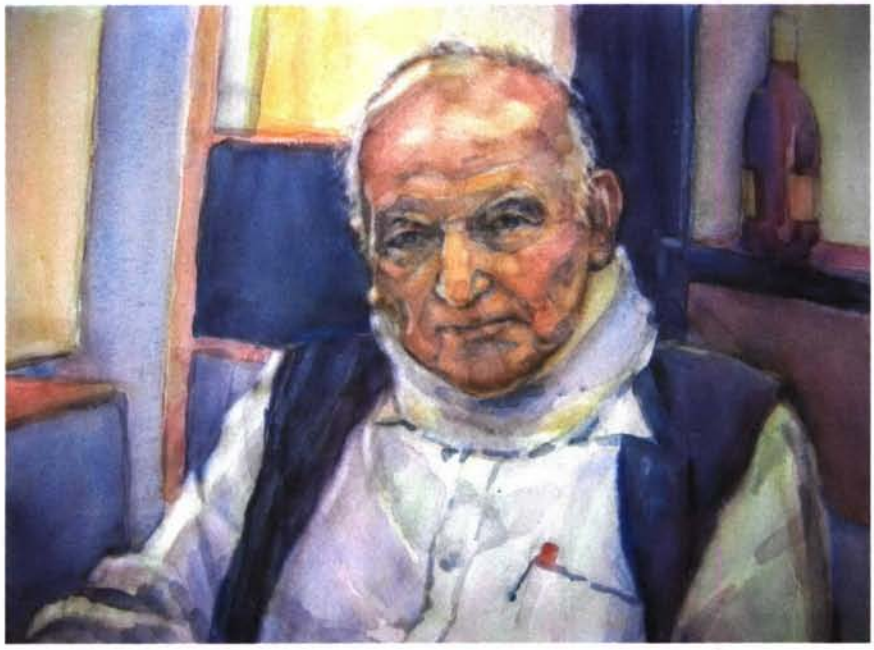
All rats in the cold-flush and control groups survived, while none of the rats in normothermic flush group had restored cardiac activity or survived. Neurologic deficit score was normal or near normal in all surviving rats and did not differ between the cold SA and control groups. Biochemical markers of organ injury were normal in all survivors. There were no differences in histologic readings of brains between survivors in hypothermic SA and control rats.

Unlike hypothermic SA, resuscitation from 21 min of ExCA with normothermic flush followed by 60 min of CPB resulted in the anticipated 100% mortality, consistent with the prolonged arrest duration. Despite this prolonged ExCA, SA followed by delayed resuscitation *via* CPB allowed intact survival, without biochemical and histological abnormalities.

The findings mirror previously published results in larger animal models signifying that the researchers have produced a rat SA model that includes 20 min of SA at 15°C with normal neurologic outcome. In light of the wealth of molecular tools available for use in rats and reduced cost of studies in rats vs. large animals, this new model should greatly facilitate and expedite the study of SA by allowing researchers to investigate the mechanisms of SA and target therapies to enhance SA. Longer duration of insult and pilot studies of promising drugs with links to hibernation-induction triggers using this new model are currently underway.

SAFAR CENTER FOR RESUSCITATION RESEARCH

2003/2004 ANNUAL REPORT



DEPARTMENT OF CRITICAL CARE MEDICINE

UNIVERSITY OF PITTSBURGH
SCHOOL OF MEDICINE

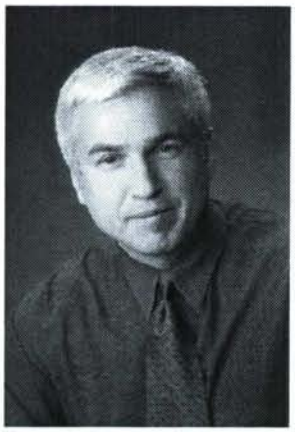
Table of Contents

Featured on the cover: Watercolor portrait of the late Dr. Peter Safar by Milly Scheffer from Buffalo, NY. This painting was created from a photograph taken of Dr. Safar on the occasion of his 79th Birthday—which occurred during the 2003/2004 academic year. The painting was presented to Mrs. Eva Safar by Dr. Larry Jenkins, Associate Director of the Safar Center for Resuscitation Research.

MISSION STATEMENT

The global mission of the Safar Center for Resuscitation Research is to improve understanding of the mechanism of secondary injury after trauma and cardiopulmonary arrest, from whatever cause, and to contribute to the development and implementation of novel therapies. The treatment and prevention of secondary injury after these life-threatening catastrophic events is a major goal in each venue of investigation.

A letter from the Safar Center's Director



Patrick M. Kochanek, MD Director, Safar Center for Resuscitation Research

The 2003/2004 academic year was another outstanding one for the Safar Center. Research. Efforts into five major areas of research and research training—including research in cardiopulmonary arrest and resuscitation, traumatic brain injury (TBI), training in pediatric neurointensive care and resuscitation research, hemorrhagic shock and suspended animation, and CNS rehabilitation research.

The most important findings generated by Safar Center investigators this academic year came from the laboratory of Dr. Robert Clark, working on a project within the cardiopulmonary arrest program, studying experimental pediatric cardiac arrest. Bob's group carried out an important study demonstrating innate gender-based effects on necrotic and apoptotic cell death pathways using a unique neuronal culture system that separately evaluated the response of XX and XY neurons to nitrosative stress, excitotoxicity, and staurosporine. They found that XY neurons were particularly sensitive to nitrosative stress and excitotoxicity, while XX neurons were most sensitive to the apoptosis inducer staurosporine. This suggests that male neurons are more apt to develop necrosis while female neurons are more inclined to develop apoptosis. They further demonstrated that this difference was, at least in part, associated with the incapacity of male neurons to maintain intracellular levels of the antioxidant reduced form of glutathione. These findings were extended to an *in vivo* model of asphyxial cardiopulmonary arrest in developing rats—further supporting the observation. That unique work was reported in the *Journal of Biological Chemistry* in a manuscript authored by Dr. Lina Du. The findings were featured as a press release on the *Nature* website. The importance of this work relates to the fact that this suggests the possibility that the clinical approach to cerebral resuscitation for any form of brain injury could be very different for females and males—i.e., a different drug cocktail or therapeutic regimen may ultimately be needed. These findings also suggest that gender differences in CNS injury are important even before puberty. This work, initially supported by funds from Pediatrics Department Chair Dr. David Perlmutter at Children's Hospital of Pittsburgh recently led to the successful acquisition of an RO-1 award by Dr. Clark—expanding his portfolio to include RO-1 funded studies in both TBI and cardiac arrest. I want to personally congratulate Dr. Clark and his talented group for this important high-impact work. In addition, Dr. Clifton Callaway, director of the cardiopulmonary arrest program of the Safar Center, and investigator in the Center for Emergency Medicine successfully competed for one of the funded sites within the NIH sponsored Resuscitation Outcomes Consortium (ROC) that emerged out of the Pulse Initiative. Special congratulations to Drs. Callaway as Principal Investigator (PI) and to Sam Tisherman as Co-PI on this important national initiative. Finally, Safar Center Scientist Dr. Robert Hickey served this year as the Vice Chairperson of the Emergency Cardiovascular Care Committee, American Heart Association. Dr. Hickey has served on that committee for a number of years, and has considerable experience, particularly in the

area of pediatric resuscitation guidelines. Congratulations to Bob. I look forward to great things from the cardiac arrest program on both the pediatric and adult fronts.

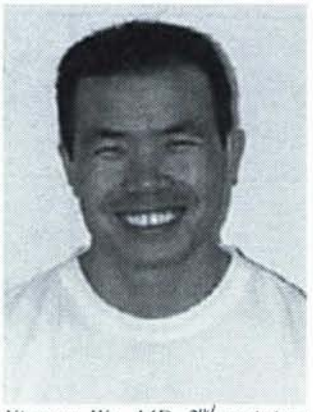
Our TBI program continues to be funded by a program project from the National Institute of Neurological Disorders and Stroke (NINDS), 9 RO-1 awards, an R-21, 2 KO-I, and 1 K23 award, participation by two of our faculty as PIs in the CDC-funded University of Pittsburgh Center for Injury Control and Research (CIRCL) grant (directed by Dr. Hank Weiss) and a variety of other grants. Our work in TBI spans a number of areas of study—including evaluation of novel resuscitative therapies targeting neuronal death, unraveling the mechanisms of secondary injury in both experimental models and in brain injured patients, the development of novel tools to facilitate detection of potentially missed cases of child abuse, and the testing of new strategies in brain injury rehabilitation. One of the most exciting developments in the area of TBI at the Safar Center this year was the successful acquisition of an RO-1 award by Dr. Anthony Kline, a scientist in our center in the Department of Physical Medicine and Rehabilitation. Anthony's work is in the area of serotonin pathways in experimental TBI. His recent studies in this area, published in the *Journal of Neurotrauma* suggest powerful beneficial effects through pharmacological manipulation of this transmitter system—specifically via 5HT-1A receptor agonists. I look forward to new discoveries by Dr. Kline, now one of the Associate Directors of our Center, and am pleased by the link between Rehabilitation and Resuscitation that has developed at the Safar Center. Our investigators published several other notable papers in experimental and clinical TBI. Dr. Kimberley Statler, working on our T-32 grant from the National Institute of Child Health and Human Development (NICHD) (see below) published a manuscript which explored one of the mechanisms underlying the potent neuroprotective effects of isoflurane—showing that this anesthetic markedly attenuated the posttraumatic hyperglycolysis believed to occur early after injury in response to excitotoxicity. She demonstrated that the commonly used analgesic in the ICU, fentanyl—failed to protect in this paradigm. Her work garnered the cover of two issues of the journal *Brain Research*. A series of two papers by Drs. Hülya Bayır and Amy Wagner demonstrated enhanced oxidative stress in adult males versus females after severe TBI. The effect of gender was more powerful than the use of therapeutic moderate hypothermia. Dr. Bayır continues to develop as a young investigator under the superb guidance of Dr. Valerian Kagan, Professor in the Department of Environmental and Occupational Health. Dr. Wagner is now an Associate Director in our Center. Both studies were published in the *Journal of Neurotrauma* and have been cited by a number of laboratories as clinical evidence for endogenous protection against oxidative injury conferred by female gender in experimental models of CNS injury. A third paper of note was generated by Dr. Paul Shore, also supported by our T-32. He carried out a unique study that compared the effect of method of CSF drainage in infants and children with severe TBI on CSF levels of a number of biochemical markers of brain injury and other endpoints, such as intracranial pressure (ICP). Remarkably, levels of mediators and ICP were lower in the children treated with continuous versus intermittent drainage. Finally, Dr. David Adelson just completed the first phase of his randomized controlled trial of therapeutic hypothermia in pediatric patients with severe TBI—an assessment of safety and feasibility of 48 hours of cooling. We look forward to analysis of those findings. I

am pleased to say that both the work of Drs. Paul Shore and Adelson represent a response to the plea in the recently published Guidelines for the Management of Severe Traumatic Brain Injury in Infants, Children, and Adolescents for more clinical studies in pediatric TBI. I look forward to future work by both of these investigators.

Research training continues to be a key priority in our Center –including the development of both postdoctoral fellows (MD and/or PhD) and junior faculty. This also represents the most important and enjoyable part of my own efforts. Postdoctoral clinician-scientist development in our Center has been greatly facilitated by our T-32 grant from the NICHD entitled "Training in Pediatric Neurointensive Care and Resuscitation Research." Graduates of our program in 2003/2004 included Drs. Paul Shore and Mary Hartman. Dr. Shore (see above for a description of some of his work) obtained a Masters degree in Clinical Research via the clinical scholars program at the University of Pittsburgh during his training on the T-32 and was highly sought after as a young clinical investigator in pediatric neurointensive care by a number of programs. He accepted a faculty position at the University of Texas Southwestern, Children's Hospital of Dallas. Dr. Hartman performed her T-32 work prior to completing her clinical training to facilitate the acquisition of an MPH degree. She has been working with Dr. Derek Angus of the Clinical Research, Investigation, and Systems Modeling of Acute Illness (CRISMA) laboratory studying the variability in the rates of triage of critically injured children with severe TBI to tertiary pediatric centers using a unique seven state database. She is reporting a remarkably high level of failure in transfer of these children in the specific population of patients who go on to die beyond 24 hours after admission. These concerning findings could have great public health impact and Dr. Hartman has presented this work to interested audiences at several national meetings. We look forward to seeing the full manuscript in press. One of our current T-32 trainees, Dr. Mandeep Chadha, also deserves congratulations for being selected as one of the top three presenters at the Annual NCMRR/NIH Training Workshop. That conference is held annually by NCMRR/NICHD and organized by Dr. Ralph Nitkin at NCMRR. It is a fantastic conference that is highly valuable to trainees. Congratulations Mandeep! Our trainees are thriving in a number of centers nationwide, including Drs. Michael Whalen, Michael Bell, and Courtney Robertson all funded by K08 awards at Massachusetts General Hospital, DC Children's Hospital, and the University of Maryland, respectively. Drs. Statler and Nguyen—recent graduates of our T32 program have applied for K awards at the University of Utah and Baylor College of Medicine, respectively. Finally, I wish to thank Drs. Ralph Nitkin, Michael Weinrich, and Carol Nicholson at NICHD for their valuable insight and support of this exciting program. In addition to clinician-scientists training on our T-32, Dr. Margaret Wilson has been working as a postdoctoral fellow in the laboratory of Dr. C. Edward Dixon, a highly respected experimental TBI scientist at our center. Margaret is carrying out studies examining striatal injury in the controlled cortical impact model and has been characterizing the Fluro-jade B method in that model.

Research productivity by the trainees continues to be spectacular, including a total of 10 fellow first-author peer-reviewed publications and 23 abstract presentations this academic year. In honor of Dr. Nancy Caroline, one of Dr. Safar's early trainees who went on to become the mother of CPR in Israel, and later, the head of the Israeli Red Cross, we

established in 2002 the Nancy Caroline Fellow Award at the Safar Center. This award is given annually to the fellow working with a Safar Center Scientist who has made the greatest contribution to the field of resuscitation medicine. Dr. Xianren Wu received this award at the 2003 Safar Symposium (see photo). Dr. Wu is a prolific young investigator working in the Suspended Animation (SA) program. His work is discussed later in this report.



Xianren Wu, MD, 2nd recipient of the Nancy Caroline Fellow Award

Many students have performed admirable research over the years at the Safar Center, and during the 2003-2004 academic year, a number of students were involved in studies at the Safar Center. Most notably, Kathleen Sachse carried out an interesting study in collaboration with both Drs. Edwin Jackson in the University of Pittsburgh Center for Pharmacology and Dr. Kochanek. Kathleen found extremely high levels of caffeine and its metabolites in the CSF of adults with severe TBI. Importantly, increased caffeine levels in the initial 24 hours after injury were associated with favorable long-term outcome. This work suggests a possible beneficial effect of caffeine via its well known up-regulation of adenosine A1 receptor number or function—making adenosine a more potent endogenous neuroprotectant. Our laboratory had previously demonstrated marked increases in adenosine in brain early after severe TBI in humans—making this a logical mechanistic possibility. Kathleen presented this work at the 2004 American Society of Anesthesiology meeting where the report received attention by the press. Dr. Kochanek was interviewed by CBS News about this work. Finally, Kathleen also presented this work at the 2003 meeting of the National Neurotrauma Society, where she was selected as one of the poster finalists. We are pleased to report that Kathleen will be joining the Anesthesiology Residency program here at Pitt—she will be a terrific addition to the program and we look forward to working with her in the future. We thank Dr. Jackson for his continued support of our work related to adenosine in CNS injury. His input is nothing short of genius.

Junior faculty development continues to be another vital component of our work at the Safar Center. Drs. Robert Hickey in the Division of Pediatric Emergency Medicine (mentored by Dr. Steven Graham) and Amy Wagner in the Department of Physical Medicine and Rehabilitation (PM&R) (mentored by Dr. Dixon), continue to be supported by KO-8 awards, and Dr. Rachel Berger in the Department of Pediatrics (mentored by Dr. Kochanek) is supported by a K-23 from NICHD. Finally, collaborator Dr. Sam Poloyac in the School of Pharmacy was funded by a grant from the American Heart Association—Pennsylvania-Delaware Affiliate for work on cytochrome-P450 metabolism in brain ischemia. Dr. Poloyac is a promising young investigator in this area. I continue to be particularly proud of our successes in fellow, resident, student, and faculty development, which I feel is the most important facet of our work.

The hemorrhagic shock and suspended animation program thrived in 2003/2004 guided by Drs. Samuel Tisherman and Patrick Kochanek. This program, which is focused on novel approaches to resuscitation of traumatic hemorrhagic shock and exsanguination

cardiac arrest, continues to be supported through a congressional appropriation funded through the United States Army. Dr. Peter Safar was the Principal Investigator of this project until his passing on August 3, 2003. Dr. Patrick Kochanek assumed the role of Principal Investigator with the approval of the United States Army. The program is focused on new approaches to the use of hypothermia and other pharmacologic strategies for protection and preservation of the entire organism during circulatory arrest. A number of studies were carried out in the 2003/2004 academic year. Most notable is the provocative study of Dr. Xianren Wu. Prior work in our dog model of suspended animation induced by aortic flush with ~20 liters of ice cold saline demonstrated that dogs could be preserved for at least 2 hours at ~7°C after rapid exsanguination over 5 minutes to cardiac arrest. These animals could be successfully resuscitated to normal outcome using cardiopulmonary bypass to carry out a delayed resuscitation. Despite these impressive findings we were challenged by the US Army to address another important scenario—particularly relevant to combat casualty care—namely to determine if suspended animation could still be successful if a prolonged period of hemorrhagic shock preceded the arrest. Dr. Wu carried out a series of studies demonstrating unequivocally, that Suspended Animation—with normal long-term outcome—could be successful even if a hemorrhagic shock interval of 2 hours preceded the arrest. This work opens the door to the potential use of this novel modality, even in the setting where a casualty was pinned down for hours after being wounded. The potential relevance of the findings to civilian trauma is also obvious. Consultative and administrative support from Dr. Lyn Yaffe, former director of the United States Naval Medical Research Institute continues to be instrumental to the program. Dr. Yaffe is working with industrial partners to develop a novel approach to catheterization and is a vital resource and a special friend to our Center. We cannot thank him enough for this support. In addition, Mr. Dave McMurry and his team at Ardiem Medical have supported cooling device development for our studies. Dr. Miro Klain has also been working on field catheterization-related aspects of this work. We are also very thankful to COL Dean Calcagni and Mr. Robert Read of the United States Army for their continued encouragement and support at the Telemedicine and Advanced Technology Research Center (TATRC) of the United States Army Medical Research and Materiel Command. Finally, Dr. Tomas Drabek, a cardiac anesthesiologist from Prague, Czechoslovakia has just begun to work as a fellow on the suspended animation project. He is developing a rat model of SA to study mechanisms with Dr. Larry Jenkins, using proteomic methods and screen novel therapies. We welcome Dr. Drabek to our team.

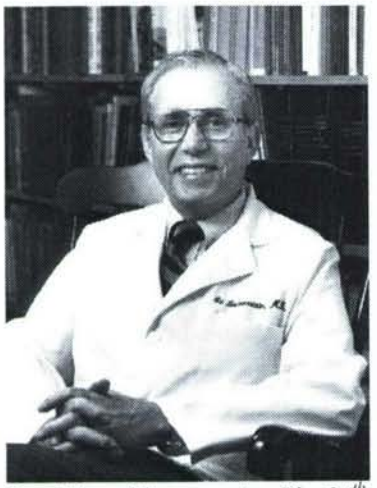
Pictured from left to right are United States Congressman John Murtha and Dr. Kochanek discussing emergency hypothermia at the ARMtech Exposition.

We would like to thank Congressman John Murtha for his support of this project in his role on defense appropriations. As the 2004/2005 academic year began, Dr. Kochanek presented

our work on this project to Congressman Murtha at the ARMtech exposition (see photo). We are indebted to Congressman Murtha for his support of our unique program.

Investigators in the Center published 29 peer-reviewed papers, 25 chapters and editorials, and 63 abstracts in 2003/2004. Included among these reports were publications in the *Journal of Neurotrauma, Journal of Cerebral Blood Flow and Metabolism, Journal of Neurochemistry, Journal of Biological Chemistry, Critical Care Medicine, Neurosurgery, Journal of Trauma,* and *Pediatric Critical Care Medicine.*

On October 30, 2003, we hosted the second Safar Symposium at the University of Pittsburgh School of Medicine. It featured sessions on *Breakthroughs in Resuscitation Research* and on the *Role of Human Simulation in Medical Education and Research.* The symposium was linked to a memorial service held in the late Dr. Safar's honor at the Heinz Chapel on the University of Pittsburgh Campus.



Dr. Edward Lowenstein. The 24th Peter and Eva Safar Lecturer

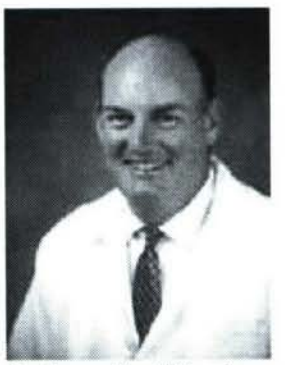
One hundred and sixty-five clinicians, scientists, and allied faculty, fellows, paramedics, and students attended the symposium. Invited speakers included Drs. John Hallenbeck, John Povlishock, Lance Becker, Donald Marion, Lyn Yaffe, Doris Ostergaard, and Mr. Tore Laerdal.

During the Symposium, we also held the 24th Peter and Eva Safar Annual Lectureship in the Medical Sciences and the Humanities on October 30, 2003. Dr. Edward Lowenstein addressed the topic of Ethics, physicians, and the relief of intolerable suffering: Lessons learned from the Oregon Death with Dignity Act. The issue of end of life care was important to Dr. Safar throughout his career, making this topic germane for the Safar Lectureship.

Pictured from left to right are Mr. Robert Read, Mrs. Eva Safar and COL Dean Calcagni during an award presentation from the US Army in Dr. Safar's honor

Immediately before the lecture, COL Dean Calcagni and Mr. Robert Read of TATRC presented Mrs. Eva Safar with a special award from the US Army, honoring Dr. Safar's special contributions to combat casualty care.

Our Visiting Professor in 2003/2004 to the Safar Center for Resuscitation Research was Dr. David Hovda of the Department of Neurological Surgery at the UCLA School of Medicine. Dr. Hovda



Professor David Hovda

addressed the important topic of optimal fuels for the brain early after injury. His group has pioneered work on a variety of alternative substrates such as ketones in experimental TBI. He is one of the top investigators linking bench to bedside via PET and microdialysis in TBI and a friend to all of us at the Safar Center. Our faculty and trainees thank him for his comments on their work.

Once again, I would like to thank everyone working at the Safar Center for a terrific job this year. I am indebted to Linda Amick, Marci Provins, Fran Mistrick, Val Sabo, and Julian Smith for their administrative and secretarial excellence. Linda and Marci are extremely dedicated to the Safar Center and its success. Linda continues to take on an increasingly greater administrative role on the business end of the Center while Marci continues to serve as our key secretarial resource for the academic programs in our Center –along with her dedicated work as my local editorial assistant for the journal *Pediatric Critical Care Medicine*. Fran Mistrick serves as the coordinator for the annual Safar Symposium, and is doing a superb job on this project, along with a number of other roles related to the archives and legacy of Dr. Safar. Fran also did a special job on the memorial service to Dr. Safar. What pleases me the most is all of the help that Linda, Marci, Fran, Val, and Julian have given to the many investigators working at the Safar Center. I would also like to personally thank Henry Alexander, John Melick, Keri Janesko, Vincent Vagni, Xiecheng Ma, Dr. Lina Du, Paula Nathaniel, Ray Griffith, Jackie Pantazes, Grant Peters, and Research Assistant Professor S. William Stezoski, who are senior administrative and technical staff members during the 2002/2003 academic year for their spectacular contributions to the individual missions of the Center. Bill Stezoski oversees the work of technicians Jeremy Henchir, Sherman Culver, and Jason Stezoski who work on our novel SA project and help it reach new levels of success. I continue to be amazed by the work ethic of all of the technical and secretarial staff at our Center.

I would like to thank Dr. Mitchell Fink for his support as the Chairman of the Department of Critical Care Medicine and Ms. Susan Stokes, departmental administrator. I am grateful to them for their support and particularly grateful to them for helping to launch the renovation that is about to begin in our center. Dr. Fink has been a steadfast leader of our center. I would also like to personally thank Drs. Clark, Dixon, Jenkins, and Tisherman for their incredible help at the Safar Center. These four faculty members have made very special contributions to the center to make my job easier and greatly enhance our success. Their close camaraderie and guidance has been incredible. I would also like to convey special thanks to Drs. Adelson, Wagner, and Kline. Their efforts in pediatric TBI and clinical and experimental rehabilitation, respectively, have made special contributions to these important niches for our center. Much thanks is also due to Drs. Bayır, Callaway, Thompson, Lunsford, Zafonte, Zhang, Yan, Klain, Graham, DeKosky, and Hickey for their efforts in the continued development of the Safar Center, its trainees, and its programs. They have been instrumental in its success. I am especially thankful to Drs. Lunsford and Zafonte for their contributions to our renovation, and to Dr. Ann Thompson who is the director of the PICU at Children's Hospital of Pittsburgh for her continued support and guidance. I also thank Dean Arthur Levine for facilitating our facility renovation and expansion. I also thank Frank Adams, Doug Schlauch, Kelly Brown, and Dick Aradine, the architects and administrators involved in this project, for

their dedication to our new facility. Finally, I would also like to thank Dr. John Williams, Chairman of the Department of Anesthesiology, for supporting the Safar Symposium and the Peter and Eva Safar Lecture.

Special thanks are also due to Dr. Edwin Jackson in the Center for Clinical Pharmacology, Dr. Valerian Kagan in the Department of Environmental and Occupational Health, Drs. Chen Ho and Kevin Hitchens and Lesley Foley at the Pittsburgh NMR Center for Biomedical Research, Dr. Stephen Wisniewski in the Department of Epidemiology, Dr. Robert Garman of Consultants in Veterinary Pathology, Inc, Dr. Timothy Carlos in the Department of Medicine, Dr. Simon Watkins in the Department of Cell Biology and Physiology, Dr. Timothy Billiar in the Department of Surgery, Dr. Paul Paris in the Department of Emergency Medicine, Dr. David Perlmutter in the Department of Pediatrics, and Dr. Samuel Poloyac in the School of Pharmacy for outstanding collaborative expertise that raises the level of the research at the Safar Center. These collaborators have been tremendous resources for our faculty and trainees, and have contributed importantly to our funding successes. I cannot thank them enough.

I also owe a debt of gratitude to Mr. Tore Laerdal of Laerdal Medical and to Mr. Hans Dahl of the Laerdal Foundation. Their generous support of our young investigators through the Laerdal Foundation has been special throughout many years. Dr. Ake Grenvik has also served as an important liaison in this regard for our Center and we thank him for his many efforts.

Finally, with the help of Chancellor Nordenberg, we continue fundraising efforts for three funds, including a "Safar Legacy Fund," to provide a core budget for the Center, along with funds to support the "Nancy Caroline Fellowship Award" and, of course, the "Safar Symposium." We have enclosed a pledge card describing those funds in this year's report and thank you in advance for your support. I would also like to personally thank each of you who have already donated to these efforts. Our total goal for these three programs is an endowment of two million dollars toward Dr. Safar's goal of the resuscitation of "brains and hearts too good to die."

I once again look forward to success in 2004/2005 in our investigative efforts to develop new therapies in the field of resuscitation medicine, and thank you for your continued support of our work.

Respectfully submitted,

Patrick M. Kochanek, MD

Patrick M. Kochanek, MD, Director, Safar Center for Resuscitation Research
Director, Traumatic Brain Injury

Clifton Callaway, MD, PhD
Associate Director, Cardiopulmonary Arrest

Robert S.B. Clark, MD
Associate Director, Molecular Biology

C. Edward Dixon, PhD
Associate Director, Functional Outcome

Larry W. Jenkins, PhD
Associate Director, Molecular Biology

Anthony E. Kline, PhD
Associate Director, Rehabilitation Research

Peter J. Safar, MD, Distinguished Professor*
Director, Shock and Suspended Animation

Samuel A. Tisherman, MD
Associate Director, Shock and Suspended Animation

Amy K. Wagner, MD
Associate Director, Rehabilitation Research

Scientists
P. David Adelson, MD
Hülya Bayır, MD
Rachel Berger, MD
Nicholas Bircher, MD
Miroslav Klain, MD, PhD
S. William Stezoski
Xiaopeng Zhang, MD

Guest Scientists
Steven DeKosky, MD
Lina Du, MD
Howard Ferimer, MD
Robert Garman, DVM
Steven Graham, MD, PhD
Kristy Hendrich, BS
Robert Hickey, MD
Sam Poloyac, PhD
James V. Snyder, MD
Stephen R. Wisniewski, PhD
Hong Qu Yan, MD

Visiting Scientists
Ernesto A. Pretto, MD
Ann Radovsky, DVM, PhD
Lyn Yaffe, MD

Fellows
Amanda Al-Khalidi, PhD
Mandeep Chadha, MD
Xiangbai Chen, MD, PhD
Tomas Drabek, MD
Melinda Fiedor, MD
Ericka Fink, MD
Mary Hartman, MD
Yi-Chen Lai, MD
Ala Nozari, MD
Paul M. Shore, MD
Margaret Wilson, PhD
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George Nikhil
Joanna Pantazes
Zachary Repanshek
Kathleen Sachse
Ryan Santos
Josh Sokoloski
Chris Stangey
Matthew Tormenti
Mike Wenger
Lauren Willard

*Founding Director
Deceased – August 3, 2003

Funding

During the 2003/2004 academic year, Safar Center investigators had a total of 47 active grants. Forty-two of these grants were extramural. The direct and indirect costs for the full award period of these grants totaled **$19,262,156** and this is plotted for the current and preceding eight academic years on the following page. The specific sources of this grant support are shown on the subsequent page. Remarkably, the Safar Center is continuing to grow and maintain a high level of extramural support. This continues to require a monumental effort by our faculty since our support is almost completely derived from extramural grants. Congratulations to the faculty for their funding successes.

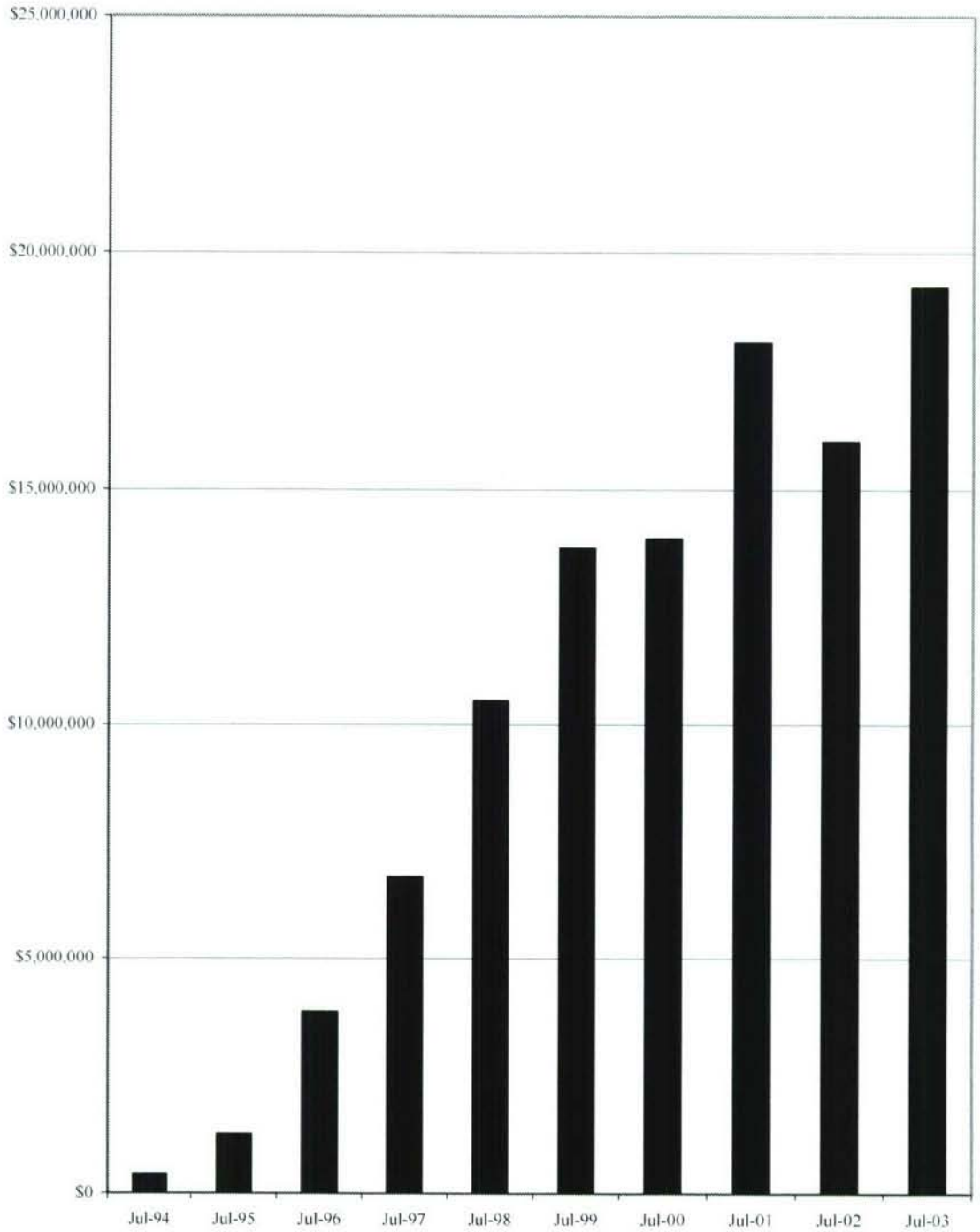
The portion of the budget for use in each academic year (July 1 through June 30) is also plotted for the current and preceding four academic years on the pages following. This represents direct and indirect costs and is shown for total, extramural, and intramural grant support.

Extramural funding sources included the National Institutes of Health, the United States Congress via the US Army, the Centers for Disease Control and Prevention, the Laerdal Foundation, and a variety of other sources, including contributions made to the Safar Center in memory of Eric Bundy.

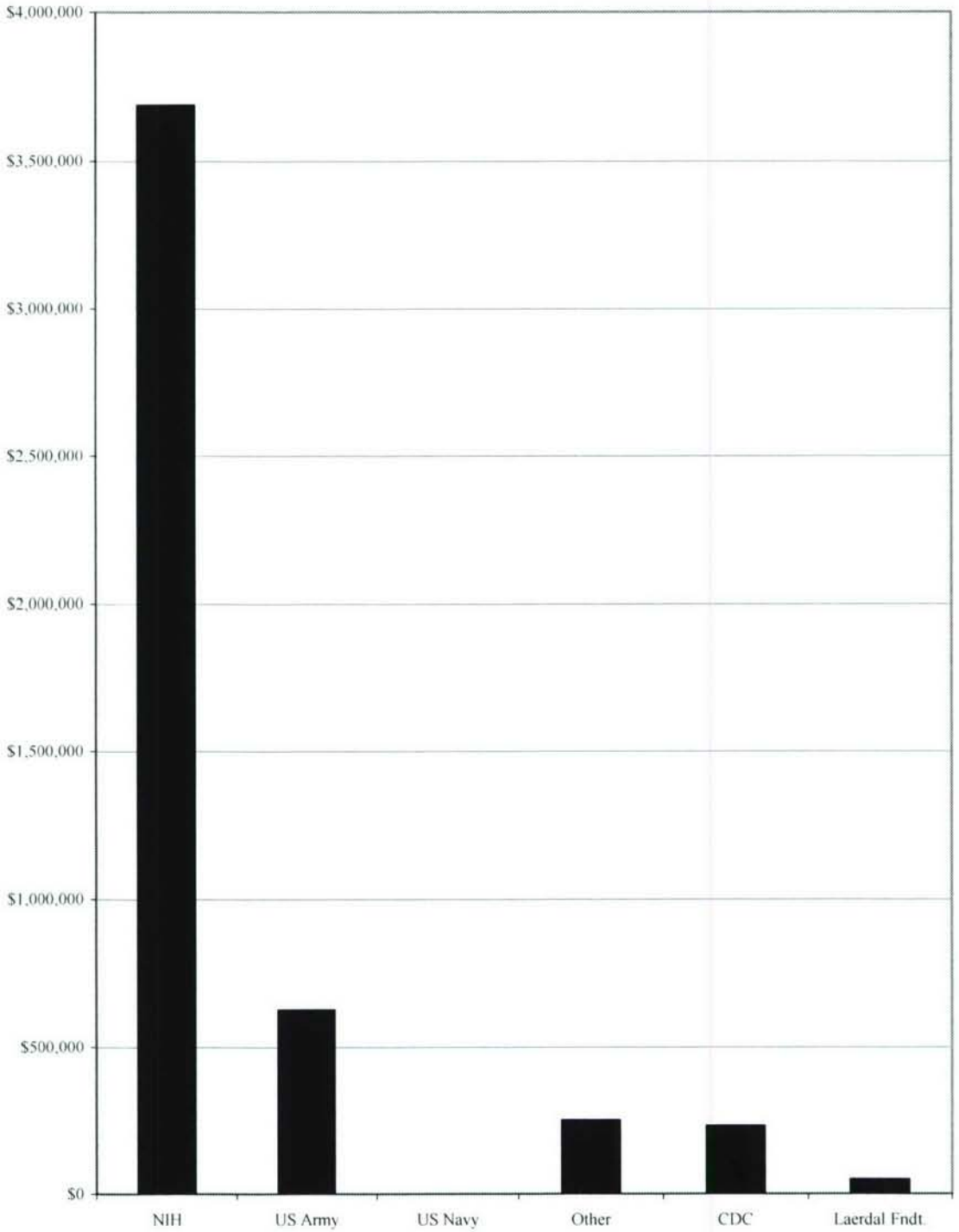
Intramural funding was provided by the Departments of Critical Care Medicine, and Anesthesiology, and the Children's Hospital of Pittsburgh.

We are deeply grateful for the prior and current support from all of these granting agencies and donors.

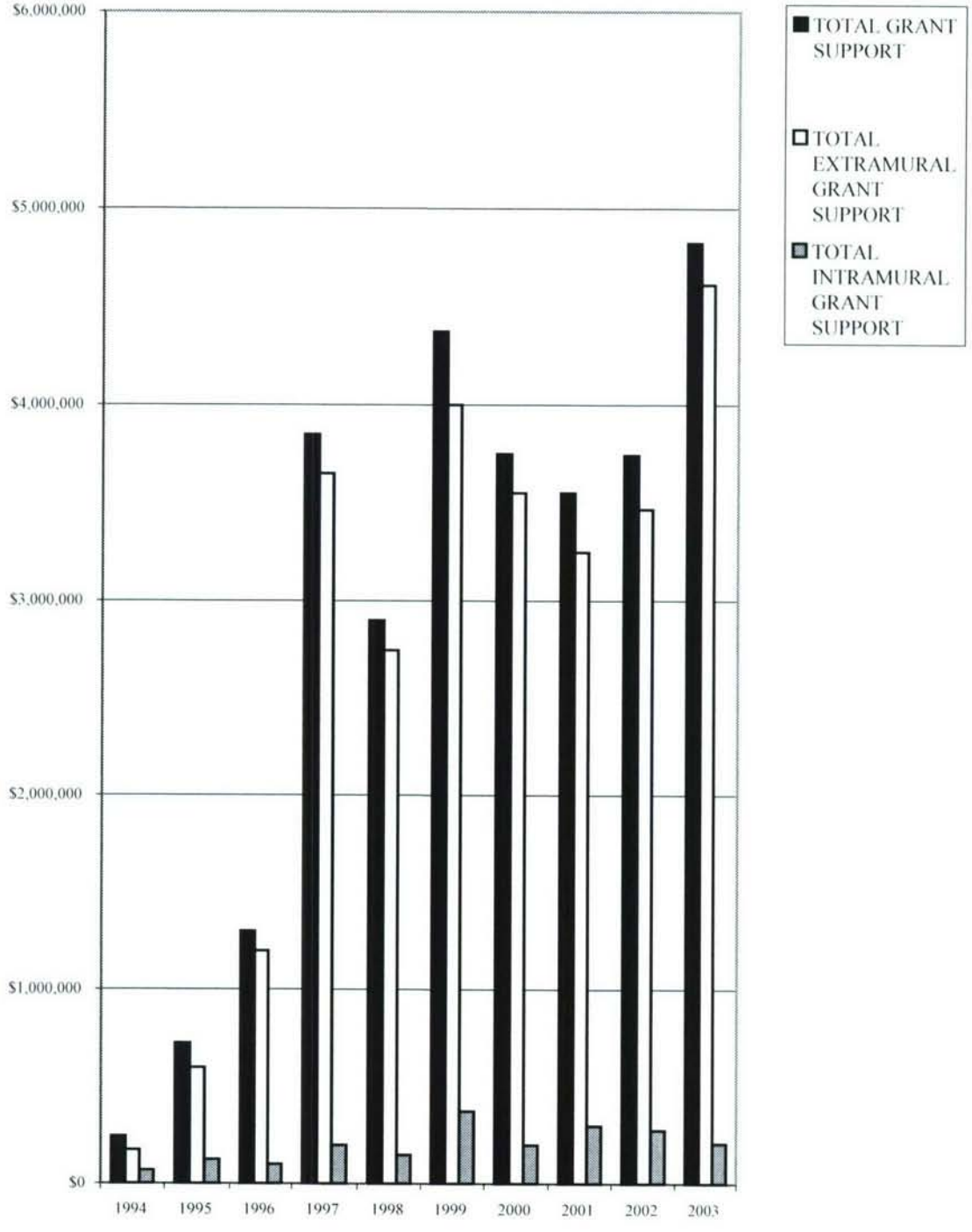
Direct and Indirect Costs for the Full Award Period of SCRR Grants



Specific Sources of Grant Support



Safar Center Grant Support through 2003/2004
use in each academic year



TRAUMATIC BRAIN INJURY (TBI) PROGRAM

Traumatic brain injury (TBI) affects 1.5 to 2 million people in the United States each year, making it one of the more prevalent and debilitating of all neurological disorders. Approximately 300,000 of the cases are severe enough to warrant hospitalization. Of the 250,000 survivors of severe TBI, 100,000 endure long-term disabilities that require rigorous, lengthy, and costly medical and rehabilitative care. In addition to the medical expenses associated with TBI, societal costs are also significant in terms of lost wages due to the inability to resume employment. While the true cost of TBI is incalculable, it is estimated at $100,000 annually per patient or about $48.3 billion per year. TBI is a serious and survivable medical problem with no acknowledged treatment. Therefore, investigation of therapeutic strategies at the Safar Center that may facilitate the recovery process after TBI are essential. Equally important are studies identifying mechanisms involved in the evolution of secondary damage after TBI and determining if pharmacological agents are detrimental to the recovery process

TBI Investigation by Safar Center Director and Associate Directors

1. Studies directed by Patrick M. Kochanek, MD

Patrick M Kochanek, MD, Director, Safar Center for Resuscitation Research, Professor and Vice Chairman, Department of Critical Care Medicine, University of Pittsburgh School of Medicine, and Professor of Anesthesiology and Pediatrics.

Dr. Kochanek's Research at the Safar Center is accomplished through a collaborative effort between a number of investigators, fellows, students and staff located principally in the Department of Critical Care Medicine (CCM), Neurosurgery, PM&R, and Neurology at the University of Pittsburgh School of Medicine. A number of collaborations are also ongoing with investigators in other University of Pittsburgh Departments including the Center for Clinical Pharmacology, Environmental and Occupational Health Medicine, Pediatrics, Epidemiology, Anesthesiology, and Surgery. A long-standing collaboration is also in place with the Pittsburgh NMR Center for Biomedical Research at Carnegie Mellon University. We have also had, this year, several important extramural collaborators, including Dr. Neal Thomas at Penn State Children's Hospital, Dr. Jiang-Fan Chen at Boston University, and Dr. Jurgen Schnermann at the National Institutes of Diabetes and Digestive and Kidney Diseases. These collaborations have allowed us to investigate a spectrum of mechanisms that may be important to the evolution of secondary damage after TBI. Our studies of mechanism of secondary damage and repair in human materials (CSF, brain tissue samples from resected contusions, and microdialysis samples) have generated new insight into the biochemistry and molecular biology of human head injury. Based on this mechanistic work, we are currently testing novel therapies in our experimental models. Our goal is to develop new therapies that can be translated to clinical application. Our clinical research taking the bench to the bedside has been featured many times in the lay press. As outlined in the opening letter,

this year, our work on caffeine levels in CSF after severe TBI in adults was featured in the national news media.

A. Biochemical Assessment of Secondary Mechanisms of Injury and/or Repair after Severe TBI in Infants and Children: The Role of Child Abuse

This continues to be an important area of research for our group and, as indicated above, continues to generate considerable publicity. We are using samples of CSF and blood collected from infants and children suffering severe TBI to study a variety of biochemical mediators of secondary damage and/or repair. These samples are collected by Dr. Rachel Berger in the Department of Pediatrics, and members of our critical care team including Drs. Clark, Bayır, Shore, Lai, Chadha, and Fink, and Dr. Adelson in the division of Neurosurgery at Children's Hospital of Pittsburgh. Dr. Kochanek is funded by the CDC (University of Pittsburgh Center for Injury Control and Research [CIRCL]) to generate this CSF repository. We have over 1,000 samples from over 100 infants and children who have suffered a severe TBI—including victims of inflicted TBI (shaken baby syndrome). We continue to collaborate with Dr. Neal Thomas at the Penn State Children's Hospital, Hershey, PA, who is also collecting samples.

Studies using the pediatric CSF bank at the Safar Center

Pediatric and adult CSF studies have produced several interesting findings in the area of TBI at the Safar Center in the 2003-2004 academic year. Work continues in a number of areas as outlined below, and in the report on Dr. Rachel Berger's investigation.

Oxidative stress in TBI

Oxidative injury is believed to be a fundamental pathway in mediating secondary damage after both ischemic and TBI. We believe that it is a key target for future development of new therapies in CNS injury. This area of study is overseen by Safar Center Scientist Dr. Hülya Bayır, a former fellow who has now joined our PICU faculty. Dr. Bayır was mentored in this area by Dr. Valerian Kagan, an international authority in free radical biology and has worked closely with him to carry out a number of studies. This year, building on a strong investigative base in this area, Dr. Bayır demonstrated a powerful endogenous antioxidant protective effect of female gender after severe TBI. A marked increase in the lipid peroxidation marker F2-isoprostane was seen exclusively in adult males after severe TBI. The gender difference was dramatic enough that it dwarfed the attenuating effect of therapeutic hypothermia on lipid peroxidation. These findings, along with the work of Dr. Wagner (see later in this report) support the possible need for gender specific therapy in TBI in adults, and specifically suggest that novel therapies targeting this mechanism are likely to be more efficacious in males. We hope to see Dr. Bayır apply novel lipidomics methods to this area in the near future in a continued collaboration with Dr. Kagan.

Adenosine in TBI

Dr. Kochanek's group is beginning experiments funded by an RO-1 from NINDS that was just successfully renewed in the area of adenosine and TBI. Translational work continues to be an important part of this effort and the CSF bank represents a key resource. Last year, we reported that T-32 fellow Dr. Paul Shore reported at the 31st Congress of the SCCM increased levels of vascular endothelial growth factor (VEGF) in CSF after severe TBI in children. The elaboration of VEGF is stimulated in part via adenosine receptor activation and local tissue hypoxia. This year, Dr. Shore published that work as a full paper in the journal Neurosurgery. Dr. Shore's work thus builds on our studies of the adenosine pathway and provides additional support for an early regenerative phase in clinical TBI. A second clinical project in this area that has created a great deal of excitement is the work of medical student Kathleen Sachse who has been collaborating with Dr. Edwin Jackson to assess the levels of caffeine and its metabolites in the CSF of adults with severe TBI. Kathleen observed remarkably high levels of caffeine in CSF of many adults with severe TBI and surprisingly, an association between CSF caffeine concentration and favorable long-term outcome. Kathleen presented this work at the 2004 meeting of the ASA where, as previously discussed, it garnered some media coverage. This potential beneficial effect of caffeine could be related to up-regulation of A1 receptor number or function—making endogenous adenosine a better neruoprotectant after injury. A beneficial effect of caffeine has recently been reported in Parkinson's disease that may be mediated via caffeine's inhibition of the A2a receptor. Further investigation is now underway in our laboratory.

CSF markers of neuronal death in TBI

For a discussion of these findings, please see the report of Dr. Robert Clark's group later in this annual report.

Effects of therapeutic hypothermia on the response to severe pediatric TBI

As Dr. David Adelson's clinical trials of therapeutic moderate hypothermia in pediatric TBI progress, we have been taking advantage of this important clinical investigation by studying CSF samples drained from the subgroup of patients at Children's Hospital of Pittsburgh. Last year, Dr. Bayır demonstrated that hypothermia attenuated oxidative injury after TBI in children. This year, she surprisingly showed no effect of hypothermia on nitrotyrosine levels in CSF in children after severe TBI. This suggests a differential effect of hypothermia on oxidative vs nitrative stress in patients. That work was presented at the 33rd SCCM Congress. In addition, Dr. Paul shore reported that therapeutic hypothermia (versus normothermic treatment) failed to attenuate increases in adenosine and purine-related metabolites, the cytokines IL-6 and IL-8, and several growth factors. That work, presented at the 2003 National Neurotrauma Society Meeting, further supports the concept of a differential effect of hypothermia on selected mechanisms of secondary damage and repair after severe TBI—most notably, oxidative stress, excitotoxicity and selected aspects of inflammation.

Effect of modes of CSF drainage

Dr. Paul Shore, in collaboration with Dr. Neal Thomas at Penn State Children's Hospital and Dr. David Adelson at Children's Hospital of Pittsburgh, completed and published an interesting descriptive study assessing the effect of continuous versus intermittent CSF drainage on mediator levels and pathophysiology after severe TBI in infants and children. These two approaches to CSF drainage in the treatment of intracranial hypertension have not been compared, and are used at the discretion of the treating physician/institution. Dr. Shore, reported that CSF levels of essentially all mediators tested were substantially lower in patients treated with continuous versus intermittent drainage. In addition, the amount of CSF drained in the continuous group was considerably greater than in the intermittent group and ICP was lower in the children treated with continuous drainage. This study was published by Dr. Shore in the *Journal of Neurotrauma* and suggests the need for a prospective clinical trial to compare these two approaches to treatment since dramatic differences were seen in this preliminary clinical trial.

Our pediatric CSF repository continues to represent a key research tool of our trainees to help bring the bench to bedside in the study of secondary injury mechanism in clinical TBI research.

Support: Improving the Diagnosis and Prognosis of Inflicted Head Trauma in Infants R49/CCR310285-03, (9/1/03-8/31/08), $139,163 (DC $95,265 and IDC $45,898), P Kochanek, PI, M Heyes, PhD, [Curagen Corporation], R Berger, S Wisniewski, PhD, and P David Adelson, MD, Co-investigators); collaborators. CDC, CIRCL (H Weiss, PhD, PI); Adenosine and TBI, NS38087, $311,420 (DC $213,749 and IDC $97,671), P Kochanek, PI; iNOS and TBI, NS30318 (P Kochanek, PI), Project 3 in the University of Pittsburgh Brain Trauma Research Center (BTRC), CE Dixon, PI. Protocol #3480500 (3/1/03-2/29/04), $179,434 (DC $122,525 and IDC $39,047), R Berger, PI, CHP GCRC. Oxidative Stress after Severe Head Injury in Infants and Children: Effect of Therapeutic Hypothermia, Laerdal Foundation, (01/01/03-12/31/03), $11,975, H Bayır, PI.

B. Adenosine and TBI

Adenosine is produced during the breakdown of adenosine triphosphate (ATP) after TBI. Its powerful vasodilator, anti-excitotoxic, and anti-inflammatory effects may represent an important endogenous defense mechanism in injured brain. The role of adenosine after TBI is being pursued both in the rat TBI model and in patients after TBI. This program includes both bench and bedside investigation, as discussed above with the clinical CSF studies. In the laboratory, we are completing studies examining the effects of adenosine agonists and antagonists on CBF as assessed by MRI. Dramatic and sustained increases in CBF were observed. This is discussed further in our section on MRI and TBI. We have also begun to carry out studies in two important knockout (ko) mice – namely the A2a receptor ko provided by Dr. Jiang-Fan Chen at Boston University, and the A1-receptor ko mouse supplied by Dr. Jurgen Schnermann at NIDDK. This project continues to be the most active area of research in Dr. Kochanek's laboratory this year and is being

carried out in collaboration with Dr. Edwin Jackson in the Center for Clinical Pharmacology

Support: NIH RO-1, Adenosine and TBI, NS 38087-05, (8/1/03-7/31/04), $311,420 (DC $213,749 and IDC $97,671), P Kochanek, MD, PI, National Institute of Neurological Disorders and Stroke (NINDS) and Dr. Shore is supported by NIH T32, Pediatric Neurointensive Care and Resuscitation Research, T32-HD40686, (5/1/03-4/30/04), $249,304 (DC $232,195 and IDC $17,109), P Kochanek, PI, National Institute of Child Health and Development (NICHD).

C. Role of Inducible Nitric Oxide Synthase (iNOS) in the Inflammatory Response after TBI

iNOS is induced by cytokines and NF-κB is suggested to play an important role in the pathophysiology of sepsis outside of the central nervous system. Both beneficial and detrimental actions of iNOS have been reported. Using both inhibitors of iNOS and knockout mice, Dr. Elizabeth Sinz (1996-97 Charles Schertz Fellow) reported a powerful endogenous neuroprotectant effect of iNOS in experimental TBI. New faculty member Dr. Hülya Bayır, in collaboration with Drs. Kagan and Timothy Billiar, has been carrying out studies to define some of the endogenous neuroprotectant effects of iNOS. Her preliminary work suggests an important endogenous antioxidant effect of iNOS-derived NO *in vivo* and was presented at the 2003 meeting of the National Neurotrauma Society. Dr. Bayır is also examining the contribution of iNOS to nitration and nitrosylation in brain after experimental TBI using iNOS ko mice. She is also investigating the potential roles for iNOS in post-translational modification of proteins, and immunomodulation. This area of study is carried out as part of our funded project within the University of Pittsburgh Brain Trauma Research Center (BTRC) Program Project.

Support: NIH 2P50 NS30318, iNOS and TBI, $179,434 (DC $122,525 and IDC $39,047), P Kochanek, MD, PI, Key Collaborators: H Bayır, MD, RSB Clark, MD, CE Dixon, PhD, T Billiar, MD, V Kagan, PhD, L Jenkins, PhD, X Zhang, PhD, and T Carlos.

D. Emergency Interventions after TBI: Effect on Secondary Damage

Last year, work was completed on this project that was focused on the assessment of the effects of the administration of various anesthetics and sedatives early after experimental TBI in rats. We are now publishing the findings. Previously we reported powerful neuroprotectant effects of isoflurane versus fentanyl in rats anesthetized with these agents at the time of trauma. We also carried out a study comparing 7 different sedative/analgesic regimens—applied early after injury and again showed that isoflurane was the most beneficial. This year Dr. Kimberly Statler published a study demonstrating that isoflurane powerfully attenuated the marked early increase in cerebral glucose utilization that accompanies excitotoxicity early after TBI. In contrast, increased glucose utilization was elevated after TBI in rats anesthetized with fentanyl. Figures from Dr.

Statler's work were featured on the cover of two issues of *Brain Research*. Dr. Statler (one of our T-32 fellows) has been the leading investigator on this work and is joining the faculty of the University of Utah, where she plans to continue her research. These studies suggest that isoflurane may limit excitotoxicity in experimental TBI, making it more difficult to demonstrate beneficial effects of therapies. In addition, clinically used narcotics such as fentanyl and morphine fail to provide any anti-excitotoxic protection.

Support: This work was previously funded by the US Army, the Laerdal Foundation, and Dr. Statler was supported by T32-HD40686, from the National Center for Medical Rehabilitation Research (NCMRR), National Institute of Child Health and Development (NICHD), P Kochanek, PI .

E. Magnetic Resonance Imaging (MRI) Assessment of Experimental TBI

Contemporary and novel MRI methods are being used to characterize our injury model and facilitate the testing of novel therapies in experimental TBI in rats. The goal of this work is to use non-invasive NMR methods to access acute physiologic derangements early after injury and to couple these to assessment of functional outcome at more delayed times after TBI. MRI methods were used to augment investigation in our study of both adenosine (see above) and anesthetics in experimental TBI. Our work examining the effects of adenosine agonists on CBF in normal rats and in rats after experimental TBI is in preparation for publication. We have begun to use MRI to assess CBF in our mouse CCI model with the help of Kevin Hitchens and Lesley Foley. Our initial studies show feasibility of this method in mice and we look forward to the first full study of this application. Dr. Ho's outstanding multidisciplinary NMR Center for Biomedical Research continues to be a key collaboration for our work in experimental TBI.

Support: NIH-NINDS 2P50 NS3031809 A1, Rat/Surgery/Imaging Core C, $192,628 (IDC $131,534 and DC $61,094 - $655,696 over 5 years, P Kochanek, MD, PI, C Ho, PhD, Co-PI, Kevin Hitchens, Lesley Foley, and Edwin Jackson, Co-investigators). NIH Grants RR-03631 and RR-10962, (C Ho, PI) support the Multidisciplinary Pittsburgh NMR Center at Carnegie Mellon University. NIH PAR00-031, In-Vivo MR Microscopy Instrumentation at 11.7 Tesla ($500,000, C Ho, PhD).

Miscellaneous

Dr. Kochanek served as one of the four editors of the new edition of the Shoemaker Textbook of Critical Care Medicine--a huge project that has just gone to press. He also continues to serve as the Editor in Chief of the journal *Pediatric Critical Care Medicine*. Finally, he authored or oversaw the preparation of a number of chapters in books in the area of experimental and clinical TBI and pediatric neurointensive care, including a comprehensive chapter on the topic of hypothermia in experimental and clinical TBI that will be published in a new book co-edited by Drs. Samuel Tisherman and Fritz Sterz on Therapeutic Hypothermia. Other chapters are included in the bibliography of the TBI program.

2. Studies directed by C. Edward Dixon, PhD

C. Edward Dixon, PhD, Professor of Neurological Surgery, Anesthesiology, Neurobiology, and PM&R, University of Pittsburgh School of Medicine. Director, University of Pittsburgh Brain Trauma Research Center

Research Interests

Research in Dr. Dixon's laboratory is directed towards understanding the molecular mechanisms of cognitive deficits following TBI. Current studies are evaluating the effects of brain injury on dopaminergic and cholinergic systems and the relationship between these changes and the induction and recovery cognitive deficits. Experimental neurotherapeutic studies are ongoing to evaluate the effects of neurotrophic growth factors and neurotransmitter receptor activation on recovery of function. Clinical studies include measuring CSF and extracellular levels of catecholamines and markers of oxidative injury in humans acutely after brain trauma. Dr. Dixon also collaborates closely with many of the Safar Center investigators as the director of the functional outcome core facility for experimental TBI research.

A. Dopaminergic/Cholinergic Mechanisms of TBI

Recovery of cognitive function after TBI is a dynamic process in which alterations in neurotransmitter systems do not likely occur in isolation. Prior work in our laboratory demonstrated that substantial cholinergic neurotransmission deficits occur without a chronic (4-wk post injury) loss of cholinergic cell bodies and that that TBI causes chronic changes in key dopaminergic proteins that occur concomitantly with these cholinergic changes. Numerous studies have shown that the dopaminergic innervation of medial septum and diagonal band of broca (medial septal area [MSA]) regions that are dense with cholinergic neurons, can affect hippocampal acetylcholine (ACh) release, especially via D1 receptor agonists. Furthermore, our data suggest that dopaminergic innervation of cholinergic nuclei is reduced after TBI. In this project, our hypothesis is that cognitive deficits after TBI may be, at least partially, attributable to decreased dopamine (DA) modulation of septohippocampal cholinergic function. A systematic series of studies are testing this hypothesis. Our focus is on DA modulation of the selectively vulnerable septohippocampal cholinergic system. To better grade an effect of TBI on these systems, we will compare in the MSA the effects of TBI to an established model of DA deafferentation effects; 6-hydroxydopamine (6-OHDA)-induced DA denervation. We will examine the effects of TBI and 6-OHDA lesions on DA modulated ACh release in the hippocampus and DA release in the medial septum. We will also determine whether changes in hippocampal ACh release are associated with altered D1 receptors in the MSA. Dr Dixon's group will determine the effect of exogeneous administration of neurotrophic factors on DA biochemical markers, cognitive deficits, as well as hippocampal ACh release and MSA DA release after TBI. Lastly, we will determine the effects of clinically relevant DA agonist therapies on cognitive deficits, as well as

hippocampal ACh release and MSA DA release after TBI. Our long-term goal is to develop new therapies to accelerate cognitive recovery following TBI.

During this year, we reported at the National Neurotrauma Society meeting that DA transporter expression was reduced in striatum after TBI in rats. Similarly, in collaboration with Dr. Amy Wagner, we demonstrated effects of environmental enrichment on frontal cortex DA transporter and BDNF. Those results are discussed in greater detail in Dr. Wagner's section. Finally, in related studies, postdoctoral fellow Margaret Wilson has been working on striatal injury in the CCI model, under the direction of Dr. Dixon, and gave presentations this year related to this work to both the Society for Neuroscience and National Neurotrauma Society. Dr. Wilson has been using Fluro-Jade B staining to study neurodegeneration in the rat CCI model and reported on both the time course of injury and the lack of effects of 8-OH-DPAT therapy on neurodegeneration after injury.

Support: NIH-NINDS, Chronic Changes in Neurotransmission Following TBI, R01 NS-33150-06 ($1,000,000/$484,819 over 5 years, 4/1/00-3/31/05, CE Dixon, PhD, PI).

B. Functional Outcome Core

The Functional Outcome Laboratory Core Facility provides a centralized site and highly standardized procedural control for all animal experiments employing functional outcome as an endpoint following TBI to rats. The Functional Outcome Laboratory Core gives the investigators of the University of Pittsburgh BTRC the capability to assess the effects of physiological manipulations and therapeutic interventions of recovery of function after experimental brain injury.

During this year, the Functional Outcome Core has evaluated post-injury function in several hundred rats and mice for seven different Principal Investigators associated with the Safar Center. This included important contributions to publications from the labs of Drs. DeKosky, Kline, and Wagner, along with a number of additional preliminary abstract reports.

Support: NIH, BTRC Supplement—Functional Core to P50 NS-30318-041A ($274,583 over 4 years, 4/1/96-3/31/00, CE Dixon, PhD, PI).

C. Examination of the Cellular Mechanisms of Mesocortical Dopaminergic Deficits after TBI in a Rodent Model Using Biochemical Indices of DA Autoxidation and Biochemical, Molecular Biological and Immunohistochemical Indices of DA Metabolism and Neurotransmission

The goal of this project is to examine the cellular mechanisms of mesocortical dopaminergic deficits after TBI in a rodent model using biochemical indices of DA autoxidation and biochemical, molecular biological and immunohistochemical indices of DA metabolism and neurotransmission. Neurochemical and immunohistochemical

markers of DA neurotransmission in the dopaminergic ventral tegmental/forebrain systems, as well as functional deficits, will be assessed after injury. The effects of therapies that either reduce oxidative damage of DA terminals and/or chronically stimulate DA activity on neurochemical and immunohistologic markers, and on functional performance will be assessed following TBI. Lastly, the relationship between early biochemical markers of DA activity to neuropsychological outcome measures specific to frontal lobe function will be evaluated in severe TBI patients. This project represents the first systematic examination of the mechanisms of induction and recovery of catecholaminergic cognitive deficits after TBI. Our long-term goal is to develop new therapies to attenuate the induction and enhance the recovery of DA-mediated neurobehavioral deficits after TBI. Some of this work has been carried out in collaboration with Dr. Amy Wagner, and details of that work are reported later in this annual report.

Support: NIH-NINDS, Mechanisms of Prefrontal Dysfunction Following Brain Trauma, R01 NS-40125-01 ($800,000/$376,775 over 4 years, 3/1/00-3/31/04, CE Dixon, PhD, PI).

D. Transcriptomic Analysis of Therapeutics in Brain Trauma

Recovery of cognitive function after TBI is a dynamic process that likely involves multiple neural systems. Several studies by our laboratory and others indicate that cognitive recovery can be enhanced by post injury activation of dopaminergic systems or exposure to an enriched environment. The effectors of such therapeutic activation are likely to involve simultaneous gene expression changes in numerous neural systems. The recent development of DNA microarrays has allowed scientists for the first time the ability to observe thousands of gene expression changes in parallel. While there are limitations, DNA microarrays provide a new systemic view to study brain injury and the treatments that stimulate and enhance recovery of function. We have evaluated a number of DA agonists that are clinically used "off label" for their ability to enhance recovery of cognitive function in our experimental model of TBI and found three to be beneficial: amantadine hydrochloride, bromocriptine, and methylphenidate. While all are putative DA agonists, they have varying degrees of specificity. We have also observed that bromocriptine treatment, when initiated 24 h after TBI, can attenuate hippocampal cell death and lipid peroxidation. This suggests that DA agonists may have mechanisms of action beyond just being DA replacement therapies (e.g. cell survival effects). Supporting this concept, our preliminary microarry data suggest that relative to a vehicle treatment, the DA agonist methylphenidate can enhance the gene expression of DA receptors and alter injury-induced inflammatory responses. DNA microarrays are well suited to investigate the effects of DA agonists on multiple pathways. The overall goal of the project is to determine common genes that are changed by these therapies and whether these gene expression changes can be further enhanced by the addition of enriched environment therapy. This project will obtain the information needed for a larger-scale R0l study to increase the number of cases, refine and increase the number of genes analyzed, and to comprehensively study those genes whose expression are related to

recovery of function after TBI. These studies of message also dovetail with the proteomic work being carried out in the lab of Dr. Larry Jenkins in the Safar Center.

Support: NIH-NINDS, R21 NS47919, Transcriptomic Analysis of Therapeutics in Brain Trauma. 07/01/03–06/30/06. $95,000-annual direct costs. CE Dixon, PhD, PI.

E. Miscellaneous

Several other areas are being investigated in the Dixon laboratory including novel studies on the effect of acupuncture treatment on functional recovery in the rat CCI model. Those studies are being carried out by Dr. Hong Yan. Similarly, preliminary work has begun in two areas, one focused on the interaction between the adenosine and dopamine systems in experimental TBI and one on the role of calcineurin signaling in histopathological and functional outcome after experimental TBI. More on these new projects will follow in next year's report.

Finally, Dr. Dixon was involved in aiding Dr. Geoff Manley in developing a pig model of CCI. That work was presented at the 2003 meeting of the National Neurotrauma Society.

Support NIH, R21 NS47919, Transcriptomic Analysis of Therapeutics in Brain Trauma. CE Dixon PI. 07/01/03–06/30/06. $285,000 total direct costs; NIH, R01 NS40125, Mechanisms of Prefrontal Dysfunction Following Brain Trauma. CE Dixon PI. 03/01/00-03/31/04. $1,000,000 total direct costs; NIH, R01 NS33150, Chronic Changes in Neurotransmission Following TBI. CE Dixon PI. 04/01/00-03/31/05. $1,645,223 total direct costs; CDC, R49 CCR312296, CIRCL: Acute Care Core Project 1–Effects of Amantadine Hydrochloride on Functional Outcome After TBI: a Randomized, Multi-Center, Placebo-Controlled Clinical Trial; and Acute Care Core Project 2-Relationship Between Amantadine Hydrochloride Efficacy and Brain Function Using PET Imaging, CE Dixon PI. 09/01/98-08/31/02. $2,709,778 total direct costs; USAMRMC, 00-451-4360, Novel Resuscitation from Lethal Hemorrhage. P Safar PI, CE Dixon Co-I. 09/15/02-09/14/03. $712,336 annual direct costs; NIH, R21 NS40049, Protein Synthesis, Memory and Pediatric Brain Injury. LW Jenkins PI, CE Dixon Co-PI. 04/01/00-03/31/03. $375,000 total direct costs; NIH, R01 NS38087, Adenosine and TBI. P Kochanek PI; CE Dixon Co-PI. 08/02/99-07/31/03. $747,440 total direct costs; NIH, R03 HD41399, Gender Differences in DA Function after TBI. AK Wagner PI, CE Dixon Co-PI. 02/06/02 – 01/31/04. $100,000 total direct costs; NIH, K08 HD40833, DA Function in TBI and Effects of Therapeutic Intervention. AK Wagner PI, CE Dixon Primary Sponsor. 09/01/01-09/30/06. $576,165 total direct costs; NIH, R03 HD043851, Interaction of Serotonin and Cholinergic Systems after TBI. AE Kline PI, CE Dixon Co-I. 04/01/03–03/31/05. $100,000 total direct costs.

3. Studies by Robert S.B. Clark, MD

Robert S.B. Clark, MD, Associate Professor of Critical Care Medicine and Pediatrics, University of Pittsburgh School of Medicine, Fellowship Director, Pediatric Critical Care Medicine Program, Children's Hospital of Pittsburgh

A. Endogenous Neuroprotectant Gene Expression after TBI

This research focuses on the genetic regulation and execution of delayed neuronal death in selectively vulnerable neurons after TBI. We have now characterized the expression of several potential cell death-suppressor genes and their translated proteins including bcl-2 gene family members and heat shock protein 72 (endogenous neuroprotectants), as well as potential cell death-effector genes including the pro-apoptotic bcl-2 gene family member bax. These genes appear to be up-regulated and/or activated after TBI in both our experimental model (CCI injury with secondary hypoxemic insult followed by resuscitation in rats) and in humans. Studies documenting that bcl-2 family genes may be important in both adult and pediatric patients after TBI were reported previously in the *FASEB Journal* and the *Journal of Pediatrics*, respectively.

A role for heat shock proteins after human head injury is also being investigated. Regulation of some of these proteins is via post-translational modification, including the bcl-2 family members bad and bag-1. Bag-1 regulates the chaperone function of heat shock proteins, pointing to a direct interaction between these two classes of endogenous neuroprotectants. This interaction was demonstrated in human brain after injury by Dr. Neal Seidberg, a PCCM fellow, and others in the laboratory. This work was published in the *Journal of Neurochemistry*.

This year, PCCM fellow Dr. Yichen Lai carried out a study examining the stress response to TBI in infants and children and reported that the stress protein HSP-70 was markedly increased in CSF after injury, particularly (over 3-fold) in victims of inflicted TBI (child abuse). This represents another mechanistic pathway demonstrating a unique profile in the abuse victims. In this case, chronic injury or stress, among other factors, could play an important role. Dr. Lai's paper on this work was published in the *Journal of Neurotrauma*. Also, summer student, Christopher Stange studied the endogenous neuroprotectant protein HSP-60 and demonstrated increases in CSF after severe TBI in infants and children. Chris gave an outstanding presentation of that work at the 2003 Congress of the Society of Critical Care Medicine (SCCM). Similarly, summer student J'Mir Cousar, working in the University of Pittsburgh School of Medicine Summer Enrichment Program, authored an abstract on increases in heme oxygenase-1 in CSF after pediatric TBI. The Heme oxygenase pathway is well known to have important neuroprotective effects in preconditioning and related models. That work was presented at the 2003 Congress of the SCCM. Congratulations to Christopher and J'Mir for a job well done.

B. Divergent Pathways of Cell Death after Brain Injury

Increasing evidence suggests that activation of caspases regulate and execute programmed cell death after TBI in experimental models and in humans. Accordingly, the objective of this research is to develop pharmacological and molecular treatment strategies that reduce caspase-mediated programmed-cell death after TBI. We previously described potential roles for caspase-1 and –3 after severe TBI in humans in a paper published in the *FASEB Journal*. Studies examining other more potent caspase inhibitors, and combination treatment strategies targeting multiple points in the programmed cell death cascade are ongoing.

This year, to continue to bridge bench and bedside in this research area, Dr. Lai expanded upon the work of prior T-32 Dr. Margaret Satchell and reported marked increases in CSF levels of cytochrome-c in additional pediatric TBI patients. That expanded study was presented at the SCCM meeting this year and a full manuscript will follow. The release of cytochrome-c was again associated with inflicted TBI—further supporting unique facets of this injury mechanism related to secondary neuronal death—presumably by apoptotic pathways. This suggests potential unique therapeutic targets for secondary brain injury in child abuse victims. I am pleased to say that this year Dr. Lai will join our T-32 program.

It is clear that both apoptotic and necrotic cell death contribute to neuronal cell loss after acute brain injury; however, recent data suggest that this is in fact over simplistic, and that multiple, interrelated pathways exist. A key regulator in this regard is the mitochondrial protein AIF. Work by Dr. Xiaopeng Zhang under the direction of Dr. Clark has shown that AIF-mediated cell death occurs after experimental TBI. That work was published in the *Journal of Neurochemistry*. Last year Drs. Zhang and Clark demonstrated an important role for an additional pathway of delayed neuronal death after experimental and clinical TBI—namely—the Fas/Fas ligand pathway. They reported, in the *FASEB Journal*, caspase-8 expression and proteolysis in human brain after severe TBI—suggesting the need for additional experimental and clinical investigation of this pathway in TBI, and the possibility of novel avenues for therapy. Ongoing studies are determining the contribution of these divergent pathways of cell death to secondary damage in TBI using multiple strategies in collaboration with Drs. Jun Chen, Steven Graham, Patrick Kochanek, Csaba Szabo (Inotek Corp., Beverly, MA), Simon Watkins, Hector Wong (Cincinnati Children's Medical Center), and Ian Reynolds.

This year Dr. Zhang presented two papers germane to this area of work. First, he presented, at the 2003 meeting of the Society for Neuroscience, a proteomic analysis of proteins released from rat brain mitochondria after depolarization. Second, he presented at the National Neurotrauma Society meeting evidence for activation of the protein kinase B signaling pathway after experimental TBI. He is studying this important cell death-regulating pathway in brain samples from both experimental and clinical TBI in work that is being carried out in collaboration with Dr. Larry Jenkins (see Dr. Jenkins's section later in this report).

C. PARP Activation after TBI

The study of PARP in experimental TBI is an expanding area of investigation at our center. PARP is an abundant nuclear enzyme with a role in DNA repair pathways. However, in the setting of energy failure, it is suggested that excessive ADP-ribosylation of proteins resulting from activation of PARP leads to marked nicotine adenine dinucleotide (NAD) depletion and exacerbation of energy failure. Drs. Whalen, Clark, and Kochanek collaborated with Dr. Csaba Szabo (an expert in the area of PARP and sepsis at the Inotek Corporation) to study the PARP ko mouse in our model of experimental TBI. We previously reported highly significant levels of protection against functional deficits after TBI in PARP ko vs wild-type mice, and a role for PARP inhibitors in improving outcome in experimental TBI in mice. However, we also noted deleterious effects of PARP inhibitors on memory acquisition in normal mice—supporting a role for PARP in memory acquisition. Last year, we published a report showing that intra-mitochondrial PARP activation contributes to NAD depletion and cell death, both in neuronal culture and in experimental TBI—which provided novel and valuable insight into the cascade of cell death in the setting of PARP activation—a mechanism that is believed to contribute importantly to a number of important diseases in critical care medicine including CNS injury, stroke, cardiac arrest, sepsis, shock and MOF. In addition, this work further establishes the presence of PARP in mitochondria.

This year, recently graduated T-32 fellow Dr. Margaret Satchell published a full manuscript on the in vivo work evaluating PARP inhibitors in our TBI mouse model in the *Journal of Neurochemistry*. Those studies suggest interesting direct effects of PARP on learning and memory and have stimulated the evaluation of targets of mitochondrial poly-ADP ribosylation by the Clark laboratory. Initial work reporting the discovery of mitochondrial poly-ADP-ribosylation as an important post-translational modification were published in the *Journal of Biological Chemistry*. Additional studies with PARP inhibitors are underway.

Finally, Dr. Clark's laboratory contributed a number of important chapters and reviews on cell death pathways, including a chapter in the upcoming Shoemaker Textbook of Critical Care Medicine and a review article in the journal *Critical Care*.

Support: RO1-NS38620-04, Caspase-Mediated Neuronal Death after Head Injury ($584,022 total direct costs over 4 years beginning 2/1/99, R Clark, MD, PI); RO1-NS38620 competitively renewed under the new title Divergent Pathways of Cell Death after Brain Injury ($1,187,500 total direct costs over 5 years beginning 2/1/03). P01-NS30318, PARP Activation After TBI, Project 4 of the BTRC Program Project ($595,000 total direct costs over 5 years beginning 6/1/00, R Clark, MD, PI). R44 NS37985, Ultrapotent PARS Inhibitor for CNS Trauma, NIH SBRI Subcontract Csaba Szabo ($120,000, total direct costs over 1 year beginning 9/1/02).

4. Studies directed by Larry W. Jenkins, PhD

Larry Jenkins, PhD, Associate Professor of Neurological Surgery, University of Pittsburgh School of Medicine

A. Protein Kinase B and C in Head Injury

The PKB and PKC enzyme families participate in many cellular functions including protein synthesis. Hippocampal protein synthesis after TBI is critical for neuronal survival, learning and memory, and synaptic plasticity. TBI alters hippocampal protein synthesis and while improved protein synthesis enhances recovery after cerebral ischemia, this has not been examined after TBI. Pathological changes in protein synthesis mediated by dysfunction of eIF2 and eIF4 pathways after TBI may impair the initiation and fidelity of protein synthesis and injury related restorative and growth responses. Pathological changes in the phosphoinositide 3-kinase-protein kinase B (PI3K-PKB), PKC, GSK-3, mitogen activated protein kinase (MAPK) and mTOR pathways may all be involved in abnormal protein synthesis after TBI. Protein synthesis can be modified by cap-dependent (eIF4E), cap-independent (internal ribosome entry segment [IRES]), and 5'TOP-5' oligopyrimidine tract (mTOR) protein synthesis initiation. This project tests the hypothesis that improved functional recovery following TBI can occur by therapeutically activating beneficial stress related IRES protein synthesis after injury causing stress induced tolerance to secondary injury processes. Hypothermia has been shown to be one therapeutic mechanism by which protein synthesis can be manipulated and will be examined. This year we examined a number of kinase systems with and without hypothermia treatment and have documented a number of important and surprising changes. In addition to the role of protein kinases in translation control, phosphorylation also regulates gene expression via epigenetic mechanisms via post-translation modification of histones and transcription factors. Gene expression changes exert proximal control over the types of mRNA to be translated and will also be examined in this project.

Thus, the aims of this proposal are to determine fundamental kinase and chaperone protein pathways that regulate protein synthesis in relation to hypothermia treatment after TBI by examining the control of three major initiation pathways, namely, cap-dependent, cap-independent (IRES) and 5' terminal oligopyrimidine tract (5'TOP) translation. We will further examine the expression of key protein products representative of these pathways involved in recovery from injury. Protein synthesis regulation is fundamental to most cellular processes. Recent advances in understanding the complexities of protein synthesis regulation contribute to the potential for therapeutic manipulation of protein synthesis. However, the manipulation of signals controlling protein synthesis after TBI may not only affect regional injury and restorative responses, but the normal function of relatively uninjured brain regions after TBI.

Control of protein synthesis primarily occurs at the rate-limiting step of initiation. Pathological changes in protein synthesis mediated by dysfunction of eIF2 (eIF2 - rate of

translation - quantitative) and eIF4 (eIF4-mRNA selection-qualitative) pathways after TBI may impair the rate and fidelity of protein synthesis and injury repair. Protein kinases and phosphatases modulate many critical control steps in the initiation and fidelity of protein synthesis, especially the initiation steps mediated by eIF2 and eIF4 protein pathways and thus the activity of these kinase and eIF pathways can be determined in part by their phosphorylation status. Using a reproducible and clinically relevant model of controlled cortical impact (CCI) in the rat, (resulting in spatial memory dysfunction as occurs in humans, we have identified a number of important hippocampal signaling changes that affect protein synthesis initiation. Time dependent changes in PKB, PKC isoforms, PKA, GSK-3B, 4E-BP, mTOR, p70S6K, eIF4E, and eIF2a phosphorylation after TBI have been documented and will be explored further in this project. In addition, changes in regulation of the histone code and epigenetic signaling have been documented and will be further examined.

This year we presented three abstracts in this area of research. First, Dr. Jenkins presented a report at the 2003 meeting of the National Neurotrauma Society showing that inhibitory phosphorylation of glycogen synthase 3 beta was decreased after CCI in rat pups, which may play a role in the reduction of protein synthesis after TBI. Second, Dr. Mandeep Chadha, in his first year as a T-32 fellow, presented a paper at the 2004 meeting of the Society for Pediatric Research that represented the initial proteomic assessment of a delayed time point after experimental TBI in the developing rat—specifically, PND 17. The initial results suggest feasibility of this approach since 2-D gel analysis revealed that some proteins such as GFAP showed marked increases vs controls at this delayed time point. Further analysis of these samples using both 2-D gel and power blot are underway. Finally, Dr. Weimin Gao reported our initial findings in the area of epigenetic signaling in experimental TBI. Using the CCI model in PND 17 rats, he reported that histone H3 acetylation was decreased after injury—providing the first evidence of a role for this important pathway regulating transcription. That paper was presented at the 2003 meeting of the National Neurotrauma Society, and a manuscript of that work is in preparation by Dr. Gao.

Support: NIH-NINDS, PKB and PKC in Head Injury, R01 NS42648, $ 231,250 annual direct cost, 02/15/04-01/31/08, LW Jenkins, PhD, PI).

5. Studies directed by Anthony E. Kline, PhD

Anthony E. Kline, PhD, Assistant Professor, Department of Physical Medicine and Rehabilitation (PM&R), University of Pittsburgh School of Medicine

A. Protective Effects of Serotonin$_{1A}$ (5-HT$_{1A}$) Receptor Agonists Against TBI-Induced Cognitive Deficits and Histopathology

5-HT$_{1A}$ receptors (5-HT$_{1A}$R) are abundant in brain regions, such as the cortex and hippocampus, that play key roles in learning and memory and that are susceptible to neuronal damage by TBI. During the past few years, our laboratory has been investigating the effects of 5-HT$_{1A}$ receptor agonists on neurobehavioral, cognitive, and histological

6. Studies Conducted by Amy K. Wagner, MD

Amy K Wagner, MD, Assistant Professor, Department of PM&R, University of Pittsburgh School of Medicine

A. Clinical Gender Differences in TBI Pathophysiology

There is conflicting evidence as to whether there are gender differences with TBI pathophysiology and outcomes. Some studies have reported that with brain injuries of equal magnitude, women sometimes fair worse. Previous work by Dr. Wagner shows that one year after hospitalization with TBI, women have more disability. Yet several animal studies show that female hormones are neuroprotective in attenuating aspects of secondary injury such as excitotoxicity, ischemia, and oxidative stress. We completed a retrospective clinical study using the NIH funded BTRC database to identify if there are gender differences in CSF markers of TBI and if hypothermia affects these markers in a gender specific manner. Multivariate regression modeling techniques were used to show that there are gender differences with the production and time-course of a cerebrospinal fluid marker of excitotoxic injury and a marker of ischemia early after injury. Females appear to have some neuroprotection against excitotoxic and ischemic injury. However, based on this study, hypothermia appeared to reduce excitotoxic injury primarily in males. This finding may be due to an apparent "floor effect" with hypothermia in reducing excitotoxic injury in females. Ischemic injury and excitotoxicity were also linked to a marker of oxidative stress. Again there were gender differences in the relationship of ischemia/oxidative stress and excitotoxicity/oxidative stress. Females have much lower oxidative stress loads than males for a given excitotoxic or ischemic insult. These findings indicate that there may be acute clinical correlates to the early neuroprotection previously reported in studies on experimental brain trauma. A manuscript reporting this work is currently submitted for review. Another manuscript has recently been published in the *Journal of Neurotrauma*. Dr. Wagner was just funded for a project in the successful competitive renewal of the CDC/CIRCL center grant. This grant is focusing on the role of sex hormones in mediating gender differences in CSF markers of TBI and evaluating the role of acute and chronic hormone levels on neuropsychological and functional outcome, and quality of life. Collaborators include the NIH funded Brain Trauma Research Center CSF Bank [(CE Dixon (Neurosurgery), Mary Kerr (Nursing), Ava Puccio (Neurosurgery)], Anthony Fabio (CIRCL), Ross Zafonte (PM&R, Hülya Bayır (CCM), and Sarah Berga (OB/GYN Emory University).

B. Gender Specific Effects of Environmental Enrichment on Dopamine (DA) Markers and Neurotrophin Production after Experimental TBI

Environmental enrichment has been shown in a variety of animal models to improve behavioral performance and impact neural substrates affecting plasticity such as angiogenesis, neurotrophin production, gliogenesis, and dendritic sprouting. Enrichment of the housing environment also improves spatial memory after experimental TBI in male rat models. Recently we reported that 3 weeks of environmental enrichment after experimental TBI improved cognitive recovery in male but not female rats. We then

investigated the effects of gender and an enriched environment on dopaminergic markers and neurotrophin production after TBI. Using Western Blot techniques, we evaluated dopamine transporter (DAT) levels in the striatum and frontal cortex. Results showed injury related reductions in DAT protein levels both in frontal cortex and striatum for males. Females generally did not have injury related reductions. However, enriched housing post-injury did result in reductions in regional reductions for injured females.

In the second experiment, we used western blot to evaluate brain derived neurotrophic factor (BDNF) levels in the frontal cortex and hippocampus after TBI and housing in an enriched environment. In males, no enrichment or injury effects were observed with hippocampal BDNF expression, but there was a significant post-injury increase in frontal cortex BDNF expression that was not augmented by EE. Neither injury nor EE altered frontal cortical BDNF expression in females, but there was a trend for decreased BDNF expression in the hippocampus of injured females vs. sham. In contrast, there were robust increases in hippocampal BDNF expression for EE injured females compared to both sham and injured animals placed in standard housing. These results reveal significant, region-specific gender differences in chronic BDNF expression with both injury and EE that may impact enrichment-mediated improvements in cognitive recovery and responses to therapeutic interventions. Portions of the work were funded through Dr. Wagner's NIH K08 award. Some of this work will be submitted to the journal *Neuroscience*. Future work will focus on the role of sex hormones on these findings as well as continuing to explore relevant neurotransmitter systems affecting a dimorphic response to environmental enrichment with cognitive recovery. This work was presented at the 2003 National Neurotrauma Society. Collaborators include Xiangbai Chen (PM&R), CE Dixon (Neurosurgery), A Kline (PM&R), and R Zafonte (PM&R).

C. DA Kinetics and TBI

Altered DA neurotransmission is hypothesized to play a role in neurobehavioral deficits after traumatic brain injury. DA enhancing agents (DA agonists) have been shown clinically to improve aspects of mental functioning after TBI, and have been shown in multiple animal studies to improve behavioral performance. This laboratory has reported reductions in striatal dopamine transporter (DAT) protein and increases in tyrosine hydroxylase (TH) chronically after TBI. These proteins play a critical role in DA release and reuptake. However, the effects of DAT reduction and TH increases on DA neurotransmission are unknown. Fast scan cyclic voltammetry (FSCV) permits real time in vivo evaluation of DAergic kinetics. The goal of this project was to assess striatal DA neurotransmission by evaluating presynaptic striatal DA kinetics in conjunction with neuroprotein and neurobehavioral correlates after experimental TBI. We evaluated electrically evoked DA release and DA clearance kinetics 2 weeks after injury. Striatal dopamine release during bilateral electrical stimulation of the medial forebrain bundle was monitored in anesthetized rats by FSCV in conjunction with Nafion-coated carbon fiber microelectrodes. Prior to FSCV, we also evaluated rotational behavior. After FSCV, we evaluated a variety of striatal DA markers, including DAT, TH, Dopamine type 2 receptors (DRD2), and Vesicular Monoamine Transporter (VMAT). Striatal

evoked overflow of DA was lower in injured rats, versus naïve. We also showed differences in zero and first order DA clearance for injured rats as well as an increase in DAT efficiency (function) after TBI. Decreases in DAT expression were noted post-injury, despite no changes in VMAT, indicating a regulatory change in DAT concentration. Behavioral data suggested a low incidence of rotational behavior in this injury model and correlated well with bilateral changes in presynaptic kinetics and DA marker expression. Increases in DAT efficiency post-TBI provide one explanation for the potential efficacy of DAT inhibitors (DA agonists) with improving cognitive recovery. A manuscript for this work was submitted. We plan to investigate regional and post-injury time course differences in DA kinetics as well as response to acute and chronic pharmacotherapies. This work is being conducted in conjunction with Dr. A Michael in the Dept. of Chemistry, whose research focuses on electrochemical techniques and the measurement of neurotransmitters using microsensors. Other collaborators and students include CE Dixon (Neurosurgery), R Zafonte (PM&R), Joshua Sokoloski, (PM&R/Chemistry) and Zachary Repanshek (PM&R/Chemistry). This and other pilot work (see genetics section) were used to submit an NIH R01 application evaluating the role of DAT genotype in striatal neurotransmission and responsiveness to treatment with methylphenidate in a clinical population with TBI

D. The Impact of Gender & Hormonal Status after Experimental TBI

Some studies have shown that sex hormones have neuroprotective qualities in the setting of acute traumatic brain injury. However, less is known about endogenously circulating sex hormones or if particular hormone levels at the time of injury effect behavioral recovery. Recently, we reported that females appear to have a neuroprotective advantage with behavioral recovery on motor tasks performed early after injury. However, no gender differences were noted with spatial learning later after injury. A manuscript on this work was recently published in *Brain Research*. Currently, we are beginning to evaluate the role of physiological hormone replacement in female rats on behavioral recovery after TBI. Additional work will focus on how hormone manipulations affect histochemical markers of injury. Students and collaborators include Xiangbai Chen (PM&R), Michael Wenger (PM&R/Neuroscience), Lauren Willard (PM&R/ Neuroscience), CE Dixon (Neurosurgery), A Kline (PM&R) and R Zafonte (PM&R).

E. Associations between DAT Genotype, Outcome, & CSF DA Levels after Severe TBI: A Follow-up Analysis

DA pathways have been implicated in cognitive deficits after TBI. While not associated with alterations in protein structure, the DAT genotype is associated with differences in DAT protein density and development of DA mediated pathophysiological conditions. For instance, the DAT 10/10 genotype is associated with higher DAT protein levels and is implicated in the development of attention deficit disorder. Differential DAT expression presumably also affects both pre-synaptic DA release, via reverse transport, and DA reuptake. DAT regulation may have a role in DA mediated neurotoxicity acutely after TBI and play a compensatory role with DA neurotransmission chronically after TBI.

Catecholamines, including DA and its metabolites, are subject to auto-oxidation, resulting in the formation of reactive oxygen species that can contribute to oxidative stress associated with secondary injury. Prior work from this laboratory has shown reductions in DAT protein after experimental TBI. The role of DAT genotype on injury and outcome has not been studied. We hypothesized that genetic & gender related differences in DAT density would affect CSF DA production & metabolism post-TBI, through reverse transport of DA via DAT. We genotyped & collected CSF for DA & metabolite (DOPAC & HVA) analysis via HPLC for 73 patients with acute severe TBI. Mixed effects multivariate regression analyses showed an impact of DAT genotype and a trend for female gender to increase CSF DA levels. Gender impacted CSF DOPAC & HVA production without affecting DA turnover, while DAT genotype impacted DA turnover. Further, preliminary analyses suggest acute CSF DA levels are linked to functional recovery curves. Data from this project was used to submit an NIH R01 application evaluating the role of DAT genotype in striatal neurotransmission and responsiveness to treatment with methylphenidate in clinical TBI. This work is being done in collaboration with the University of Pittsburgh BTRC, Dianxu Ren (Public Health), CE Dixon (Neurosurgery), Yvette Conley (Health Promotion and Development), Robert Ferrell (Human Genetics), Sue Beers (Psychiatry), R Zafonte (PM&R), and Mary Kerr (Nursing).

Support: NIH K08HD40833, AK Wagner, MD PI, *DA Function and the Effects of Therapeutic Intervention* $622,258 beginning 2001 for 5 years (Sponsors: CE Dixon, PhD, AC Michael PhD, and RD Zafonte, DO); NIH R03HD41399, AK Wagner PI *Gender Differences in DA Function after TBI* $145,535 beginning 2002; CDC R49/CCR323155-01-1---CIRCL, AK Wagner, MD Project PI (H Weiss PhD PI Center Grant), *Evaluating the Impact of Neuroendocrine Hormones on Pathophysiology and Outcomes after TBI* $772,948; CDC CCR310285-07---CIRCL, Small Grants Program AK Wagner, MD Project PI (H Weiss PhD PI Center Grant) $10,000 beginning 2002 for *Characterization of Alterations in the Female Rat Estrous Cycle after Experimental TBI*; NIH P50NS30318 Clinical Core--*University of Pittsburgh BTRC*, CE Dixon PI; NIH Loan Repayment Program; Department PM&R, University of Pittsburgh.

TBI Investigation by Safar Center Scientists and Visiting Scientists

7. Studies by P. David Adelson, MD

A. Severe TBI in Immature Rats

Dr. Adelson's laboratory is focused on the effect of hypothermia following TBI acutely and long term on recovery. They have been examining the role of hypothermia and its effect on excitotoxicity, cell death, and synaptic recovery following experimental TBI in developing rat, studying both postnatal day (PND) 7 and PND 17 rats and demonstrated important age-related differences in the different ages at injury. His lab has been able to demonstrate the efficacy of therapeutic hypothermia on outcome as it relates to age at injury in these same developmental paradigms. Dr. Adelson and his investigative team presented work in both of these areas at the annual meeting of the National Neurotrauma

Society. He has focused his recent efforts on the CCI model of TBI. His lab is also beginning to carry out exciting studies in collaboration with Dr. Patrick Card examining reorganization of the developing brain after experimental TBI.

Support: NIH Grant No. 1 R01 NS42298, Efficacy of Hypothermia in Pediatric TBI

B. Hypothermia for Severe TBI in Children

The major goal of this project was to test the safety and efficacy of therapeutic hypothermia in children after severe head injury. This program has been funded at an R01 level by the NIH/NINDS and investigated hypothermia as a treatment of TBI in children, with a special emphasis on the development of novel methods for the initial and outcome assessment. Dr. Adelson is the principal investigator of this important multi-center study that includes 7 centers. Dr. Harvey Levin, at the Baylor College of Medicine, is a co-investigator on that study, along with Drs. Sue Beers and Tom Campbell, at the University of Pittsburgh, that is assessing long-term functional outcomes including language and speech acquisition, long-term effects of mild to moderate head injury, and a number of other collaborative and related efforts. This phase II study is now underway.

Collaborative studies of the effect of therapeutic hypothermia on a variety of biochemical and molecular mediators of secondary injury and repair are ongoing (please see prior discussion of this area in Dr. Kochanek's report) from CSF samples obtained from patients enrolled at the Children's Hospital of Pittsburgh.

Support: NIH Grant No. 1 R21 NS043293, Hypothermia for Severe TBI in Children (planning grant) and NIH Grant No. 1 R01 NS38448, Hypothermia for Severe TBI in Children.

C. Pediatric Neurotrauma Center

The Pediatric Neurotrauma Center (PNTC) was developed through the generous support of the Federation of Independent School Alumnae and serves as a web based database and is the central data core for clinical trials locally, nationally and internationally. The many projects that have been supported by the Data Center of the PNTC include the biochemistry analyses of children following TBI and following treatment with hypothermia, speech and language outcomes, mild and moderate TBI, cerebral blood flow and metabolism following TBI, to name but a few. The PNTC has also been instrumental in its support of international clinical trials serving as the data center for a multicenter TBI project in Latin America based out of Argentina and supported by the Latin American Brain Injury Consortium (LABIC).

8. Studies by Rachel P. Berger, MD, MPH

Rachel P. Berger, MD, MPH. Assistant Professor of Pediatrics, University of Pittsburgh School of Medicine and Children's Hospital of Pittsburgh

A. Use of Serum Biomarkers in the Detection of Silent Inflicted Childhood Neurotrauma

Infants who are victims of inflicted TBI are often injured on multiple occasions or brought to care many hours to days after their injury. In addition, their injury is often not recognized since caretakers rarely provide a history of trauma and the infants often do not have any external signs of trauma. In the past year, Dr. Rachel Berger, a general pediatrician working in the area of child abuse at Children's Hospital of Pittsburgh, has broadened the potential relevance of this project by studying the potential use of serum markers of brain injury with the hope of detecting otherwise unidentified brain injury in possible victims of child abuse. Rachel first showed that CSF levels of markers of neuronal (neuron specific enolase [NSE]) and glial (S-100B) death were massively increased versus control after severe TBI in infants and children—including child abuse victims. That work was published last year in the journal *Pediatrics*. Dr. Berger also published a report in the *Journal of Neurotrauma* showing that these markers of brain injury are increased in the serum in over one-third of infants and children with mild TBI—children that are often sent home from the emergency department. This study has set the stage for an assessment of the use of these biomarkers in a target population of infants in diagnostic categories that occasionally represent missed cases of inflicted TBI-such as vomiting without diarrhea, a seizure without fever, unexplained bruising, etc. A positive serum test for such biomarkers would not confirm trauma as etiology of the increase, rather it would "point to the head" and suggest to the health care provider, the need to either obtain additional history, perform a careful fundoscopic examination, or perform a cranial imaging study. That important prospective study is the centerpiece of Dr. Berger's K-23 award—from NICHD, and the project of Drs. Berger and Kochanek that is funded by the CDC-University of Pittsburgh CIRCL.

This year work continued in this important area of research. The macrophage marker quinolinic acid (QUIN) was considered as a potential marker that could provide clues to the timing of injury—i.e.—serving as a biological clock of chronic vs acute inflammation. Despite the lack of a history of trauma in 82% of children with iTBI, 100% had a peak QUIN concentration of >100 nM. There was an increase in the CSF concentrations of QUIN after severe nTBI and iTBI in children. Dr. Berger recently published that paper in the *Journal of Neurotrauma*. Higher initial and peak QUIN concentrations after iTBI may be due to severity of injury, young age, and/or delay in seeking medical care, which allows for increased secondary injury—thus, delay in presentation may not be the only reason for the marked increase in CSF QUIN in abuse victims. Another limitation of using a CSF marker such as QUIN is that in many cases of missed child abuse, CSF is not available, and the injury is mild. In contrast, a blood test showing brain injury could potentially be valuable in this setting—and this approach is

the backdrop upon which the current NIH and CDC-funded work of Dr. Rachel Berger is progressing.

Dr. Berger also presented an abstract describing biomarker levels in serum in an expanded sample of children with known TBI. That work was presented at the 2003 meeting of the National Neurotrauma Society and a full report is in preparation for the submission to the *Journal of Neurosurgery*. This study adds further support to the potential utility of biomarkers across the spectrum of pediatric TBI. Dr. Berger also presented the first preliminary report on the potential use of serum biomarker to identify brain injury in infants presenting to an emergency department who are at increased risk for inflicted TBI. That presentation was made at the 2004 meeting of the Society for Pediatric Research (SPR). Those results are the initial data demonstrating feasibility of this approach in the target population. The clinical trial of this approach in infants with nonspecific symptoms such as vomiting without diarrhea—symptoms typical of the well-described cases of missed child abuse—is underway.

In addition, Dr. Berger was the lead author of an invited review on biomarkers in inflicted TBI that was published in the journal *Child Abuse and Neglect*. This supports the considerable interest that this approach has garnered among the brain injury and child abuse communities for TBI detection.

Finally, it is important to have information on other potentially confounding neurological diagnoses in children. To this end, Tina Dulani, a study coordinator working with Dr. Berger, reported, in another abstract at the 2004 meeting of the SPR, data on biomarkers in pediatric meningitis and seizures. Biomarker levels after hypoxic-ischemic encephalopathy are also currently under study in Dr. Berger's laboratory.

Support: 1K23HD43843-01 "Using Biochemical Markers to Detect Abusive Head Trauma," R. Berger, MD, MPH, PI. General Clinical Research Center (GCRC) M01RR00084 "Using Biochemical Markers to Detect Silent Brain Injury," R49/CCR323155-01, R Berger, MD, MPH, PI. University of Pittsburgh Center for Injury Research and Control (CIRCL), and "Can We Detect Brain Injury by Looking in the Blood?" P Kochanek, MD, PI. Children's Hospital of Pittsburgh of the UPMC Health System–Faculty Start-up Grant – "The Use of Biochemical Markers to Assess Accidental and Abusive Head Trauma in Infants and Young Children." R Berger, MD, MPH, PI

Collaborators: P. Kochanek, Critical Care Medicine; P. David Adelson, Neurosurgery; Mary Clyde Pierce, Emergency Medicine, John Leventhal, Department of Pediatrics, Yale University.

9. Studies by Steven T. DeKosky, MD

Steven T. DeKosky, MD. Professor and Chairman of the Department of Neurology and Director of the Alzheimer's Disease Research Center, University of Pittsburgh School of Medicine

A. Antioxidant and Neurotrophic Response after TBI

Dr. DeKosky's laboratory studies the role of neural cells and their products in the brain's attempt at repair following TBI. The laboratory is particularly interested in the cytokine and antioxidant cascades that occur over the course of days to weeks after injury (secondary injury processes), and their relationship with the upregulation of neuroprotective proteins such as neurotrophins. The goal is to elucidate the brain's injury response and provide insight into possible therapeutic interventions that could be used in clinical settings to treat human TBI patients.

Dr. DeKosky's group has examined the timecourse of changes in antioxidant activities (catalase, glutathione and superoxide dismutase) and neurotrophins (such as NGF) expression after experimental TBI. Close temporal relationships were observed between the upregulation of NGF protein and complex changes in antioxidant enzyme activities. To further investigate the relationship between NGF and the antioxidant enzyme response, Dr. DeKosky's group examined the effect of hypothermia on the post-injury level of NGF and on antioxidant enzyme activity, and showed that in rats subjected to post-traumatic hypothermia, both NGF protein levels and catalase and glutathione peroxidase activity levels are suppressed. In an attempt to restore post-injury antioxidant enzyme activities in hypothermia-treated animals, NGF protein was infused immediately after injury, and during the course of hypothermia treatment. The study showed that NGF infusion was ineffective in restoring enzymes' activities to post-injury levels. These results suggest several possibilities. First, based on work by several laboratories, including studies by Drs. Bayır and Wagner previously discussed in this report, hypothermia may reduce oxidative stress, spare small molecule antioxidants such as ascorbate, and thus blunt the induction of antioxidant proteins such as catalase and glutathione peroxidase. Alternatively, since infusion of exogenous NGF failed to restore normal antioxidant enzyme activity after injury—other pharmacological antioxidants may be required to maximize the beneficial effects of therapeutic hypothermia in TBI. The results of these two studies were published in the *Journal of Neurotrauma* and the *Journal of Neurochemistry*, respectively.

B. Effects of TBI on Amyloid Precursor Protein (APP) Metabolism

Alzheimer's disease (AD) is a progressive neurodegenerative disorder characterized by neuronal loss in discrete brain regions and by formation of neurofibrillary tangles and beta-amyloid associated neuritic plaques. A major component of these plaques is the 42-43 amino acid amyloid beta (Aβ) peptide that is cleaved from the transmembrane region of amyloid precursor protein (APP).

One of the known risk factors for AD is TBI. Therefore, alterations in APP processing may play an important role in the pathogenesis of both TBI and AD. To better understand the relationship between TBI and AD, Dr. DeKosky's laboratory is conducting experiments using both mouse models and surgically excised tissue and CSF samples from patients with severe head injury. Collectively, these studies center on the cytokine-

related molecular cascades involved in pathological alterations in APP and Aβ production and metabolism after TBI, and the effect of therapies designed to interrupt these cascades.

The humanized Aβ mouse model of TBI

In collaboration with Cephalon Inc., Dr. DeKosky's lab has developed a colony of mice that produce detectable levels of human Aβ (the "hAβ mouse"). This mouse represents a significant advance of previous transgenic mouse models of AD in that the APP gene is under its endogenous promoter, and APP itself is produced at normal levels. This mouse is therefore particularly important for the studies of Aβ changes after TBI because 1) unlike in rats or wildtype mice that produce rodent Aβ, we are able to employ well characterized biochemical assays to detect *human* Aβ and 2) the continuous over-expression of APP as seen in transgenic mice is avoided, which is particularly important in our injury and intervention paradigms. Dr. DeKosky's laboratory is currently examining post-injury changes in APP and Aβ proteins, as well as components of a molecular cascade involving interleukin-1β, nuclear factor κB, and caspase-3 that are involved in post-injury upregulation and amyloidogenic processing of APP. Ultimately, the goals are twofold; 1) to define the pathways causing, and pathological effects of, Aβ overproduction after TBI, and 2) to assess the effect of therapies designed to prevent Aβ overproduction after TBI in mouse models, that could potentially be translated into therapeutic strategies to treat TBI patients.

This year, studies demonstrating increased expression of cholesterol transporter for ABCA1 and APP in experimental TBI were presented at the annual meeting of the Society for Neuroscience.

Studies in TBI patients

To better understand the relationship between TBI and AD, Dr. DeKosky's laboratory is examining the distribution and levels of APP and Aβ protein in surgically resected temporal cortical tissue and serial CSF samples obtained from head-injured patients. This study is the first to demonstrate AD-like Aβ plaques in freshly resected brain tissue after severe TBI. Furthermore, within hours after TBI, human temporal cortex reacts to injury with a robust up-regulation of APP in pyramidal neurons, which likely represent the main source of Aβ. This process is paralleled by increased neuronal accumulation of amyloidogenic APP fragments, as well as a marked up-regulation of apolipoprotein E in both neurons and glial cells. These observations are important for our understanding of TBI as a potential risk factor for later development of AD, suggesting a pathological cascade that involves neuronal overproduction of APP and Aβ, and glial upregulation of apoE, the latter of which has been known to facilitate Aβ deposition in AD brains. Of additional importance, the development of acute Aβ pathology after TBI is not paralleled by formation of neurofibrillary tangles (another pathological hallmark of AD), indicating that intracellular neurofibrillary changes and progression to dementia of AD can occur only after extended survival periods (i.e., months to years). This suggests a large window

of opportunity for therapeutic interventions after TBI before the onset of cellular pathology that could lead to AD dementia. Collectively, these studies convincingly show that increases in Aβ after injury result in acute AD-like pathological alterations that could be an important target for therapies that are being developed in our humanized Aβ mouse model. These clinical studies are being carried out in collaboration with the clinical TBI core within the BTRC. A full manuscript is being submitted to *Experimental Neurology*. Dr. Milos Ikonomovic is a key co-investigator on this project.

Support: Core C of 2 P50 NS30318-04A21, Project #3 in the University of Pittsburgh Head Injury Research Center (S DeKosky, MD, PI).

10. Studies by Steven Graham, MD, PhD

Steven Graham, MD, PhD, Professor and Vice-chairman, Department of Neurology, University of Pittsburgh School of Medicine; Associate Chief of Staff for Research and Director, Geriatric Research Educational and Clinical Center, V.A. Pittsburgh Healthcare System

A. Bcl-2 Family genes in TBI

Dr. Graham's laboratory studies the molecular and cellular mechanisms of neuronal cell death. In collaboration with the Safar Center, Dr. Graham's laboratory investigates neuronal death in TBI. This work is part of the University of Pittsburgh BTRC funded by NINDS. The recent emphasis of the laboratory has been the genetic mechanisms that regulate neuronal cell death. In particular, the role of genes that regulate programmed cell death, the bcl-2 and the cysteine protease family of genes, is being investigated in trauma. Recent studies in Dr. Graham's laboratory focus on the role of the Fas cell death receptor and caspase 8 in traumatic brain injury.

Support: Core C of 2 P50 NS30318-04A21, Project #1 in the University of Pittsburgh BTRC (Steven Graham, MD, PhD, PI). Technician: Marie Rose

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CARDIOPULMONARY ARREST PROGRAM

A. Clifton Callaway and the Department of Emergency Medicine

Clifton W. Callaway, MD, PhD, Assistant Professor, Department of Emergency Medicine, University of Pittsburgh School of Medicine

We continue to emphasize the fact that resuscitation from cardiac arrest must attend to both heart and brain. About one-third of attempted resuscitations result in restoration of spontaneous circulation. Therefore, improved approaches to cardiac resuscitation are needed. Only about one-quarter of patients with restoration of circulation will regain consciousness. Therefore, therapies to improve neurological recovery are also required. Without attention to both of these organ systems, overall survival from cardiac arrest is unlikely to increase.

Work this year continued to focus on the molecular mechanisms of neurological recovery after cardiac arrest using our rat model. We committed more effort to clinical cardiac arrest research. Because induced hypothermia after cardiac arrest appears to be an effective therapy for improving brain recovery, we have advocated its acceptance as therapy rather than as research. Several presentations at Grand Rounds and departmental meetings within UPMC and Mercy Health Systems were conducted for this purpose.

1. Altered Cellular Signaling in Brain after Resuscitation

Two mitogen-activated protein kinases (MAPKs) increase in hippocampus over the 24-hour period after resuscitation from cardiac arrest: the p42/p44 MAPK (extracellular-signal regulated kinase, ERK) and the Jun-N-terminal kinase (JNK). Likewise, levels of an extracellular signaling molecule, brain-derived neurotrophic factor (BDNF), increases in hippocampus at 24 hours after resuscitation from cardiac arrest. Induction of mild hypothermia (33°C) between 1 and 23 hrs after reperfusion, further increases activity of ERK and BDNF relative to normothermic (37°C) controls. We have speculated that the beneficial effects of induced hypothermia are related to these increases in ERK and BDNF activation.

In order to study the role of BDNF expression during hypothermia, antisense oligonucleotides (AO) were infused into the lateral ventricles of rats in an effort to reduce total BDNF. A sequence for the AO against BDNF was obtained from previously published studies in rats. Rats (n=6-7 per group) received intracerebroventricular infusions (0.1 nmol/hr) of AO against BDNF or of a missense control AO. After 72 hours, the hippocampus was collected, total protein extracted, and BDNF levels measured using immunoblots. We were not able to detect any change in BDNF protein level after AO. In the event that poor penetration of the AO into cells or into parenchyma was the basis for this failure, we repeated this experiment including lipofectin in the AO vehicle, and also with direct infusion into the hippocampus. Still, there was no evidence of decreased BDNF levels. In order to confirm that AO was being taken up into neurons,

fluorescein-labeled AOs were injected into separate rats. At 72 hours after injections, we were able to visualize fluorescence in hippocampal CA1 neurons. Taken together, these data indicate that this AO can be delivered to hippocampal neurons, but produces no decrease in total BDNF protein levels.

We also have examined the effects of BDNF infusions on ERK activation. BDNF was infused into the lateral ventricles of rats at a rate of 0.025 mcg/hr using osmotic minipumps. Rats (n=3-4 per group) were sacrificed after 12, 24, 48 or 72 hours. The levels of BDNF and active ERK in hippocampus were measured using immunoblotting. This type of infusion increased tissue BDNF levels somewhat at 12 hours and robustly by 24 hours. Active ERK levels change only slightly in these brains. These data suggest that prolonged delivery of BDNF has only modest effects on ERK signaling in brain.

Last year, we determined that injection of 100 μg of U0126 into the lateral ventricle specifically reduced ERK activation in bilateral hippocampi for 12 hours. ERK activity had returned to near normal levels by 24 hours. In addition, we examined the influence of U0126 injections on several transcription factors. Rats subjected to cardiac arrest, received injections of vehicle or U0126 at 30 minutes after reperfusion (n=3-4 per group). These rats were maintained at normal temperature (37°C) or hypothermia (33°C) between 1 and 24 hours after reperfusion. Hippocampal levels of the phosphorylated forms of p90Rsk, ATF2 and CREB were examined with immunoblotting. These data indicate that hypothermia increases activation of ATF2 and CREB, and that this activation is blocked by U0126. Thus, hypothermia-induced signaling through these transcription factors is ERK-dependent.

2. Effects of Resuscitation on Brain Gene Transcription

We have prepared total RNA from rats treated with cardiac arrest followed by 12 or 24 hours of normal temperature or hypothermia (33°C). Sham rats received all surgery but no cardiac arrest. This RNA was processed by the genomics core facility for hybridization to an Affymetrix U34 Rat DNA array. Results of this gene array study should become available in summer of 2004.

In parallel with this analysis, we have set up PCR-based measurements of gene transcription. Relative changes in hsp70, hsp60, hsp27, c-fos, bdnf, GAPDH and cyclophilin were measured in reverse transcribed cDNA. A scheme for quantification has been adopted using serial dilutions of cDNA and relative intensity of the PCR products on ethidium-bromide gels. Using this technique, we have been able to measure changes in bdnf transcripts. The transcript for BDNF includes four alternative exons (called exon 1-4) that are under the control of different promoters. The PCR technique was able to determine that cardiac arrest increases expression of exon 1 and exon 3. Hypothermia specifically increases expression of exon 3. Exons 2 and 4 were not altered significantly and appeared to decrease after hypothermia. These results demonstrate that the temperature regimen after resuscitation can produce very specific changes in gene expression that are probably related to effects on specific transcription factors. These

results were presented at the Society for Neuroscience Meeting and are submitted for publication.

3. Effects of Manipulation of Cellular Signaling on Brain Injury after Cardiac Arrest

Previously, we confirmed that induced hypothermia improved neurological recovery after cardiac arrest and resuscitation. However, there were no significant differences in functional outcome between U0126-treated and vehicle-treated rats. We have subsequently completed histological analysis of these rats, and determined that hypothermia markedly reduces loss of hippocampal CA1 neurons after 14 days, and that U0126-treatment does not affect this loss. These data suggest that hypothermia-induced improvements in outcomes are not dependent upon forebrain ERK activation.

A study is in progress to examine the influence of exogenous BDNF administration on functional and histological outcome after cardiac arrest. Osmotic minipumps deliver BDNF or vehicle to both lateral ventricles at a rate of 0.025 mcg /hr for 72 hours after cardiac arrest. All rats are maintained at normal temperature. To date, BDNF (n=7) and vehicle (n=10) groups do not differ in terms of 14-day survival (71% vs 70%), weight loss, or neurobehavioral scores. Brains have not yet been examined histologically. These data suggest that BDNF infusion alone is not sufficient to reverse the severe brain injury observed after cardiac arrest.

4. Vasopressin in Cardiac Arrest

We initiated a study with the City of Pittsburgh Bureau of Emergency Medical Services to study the effect of adding vasopressin (40 IU) or placebo to standard care for out-of-hospital cardiac arrest. This study employs an Exception from the Requirement from Informed Consent for Emergency Research. The first subjects were enrolled in May 2003. Approximately 150 subjects of a planned 324 were enrolled during this year. The study remains blinded. However, overall survival of these subjects does not differ from historical patients treated in Pittsburgh who would have met the inclusion criteria. Therefore, we suspect that there is no large difference attributable to vasopressin administration.

The process of notification of subjects about enrollment in this trial has provided insight into research conducted under an Exception from Informed Consent. The rate of family member completion of informed consent documents is poor. This fact appears to be related to competing activities related to end-of-life decision making rather than lack of opportunity or active resistance. Preliminary observations about this process were presented in abstract form to the National Association of EMS Physicians.

5. Out-of-Hospital Cardiac Arrest in Pittsburgh

In preparation and during the vasopressin study, we have examined the electronic recordings collected by automated external defibrillators (AED) used by firefighter first-responders. Analysis of these recordings reveals several systematic problems with the conduct of resuscitation that may be amenable to improvement. First, there is a low

incidence of ventricular fibrillation that is converted to a perfusing rhythm by AED shocks. Second, rhythm analysis and repeated rescue shocks consume a lot of time that is not devoted to chest compressions. Finally, the ratio of 15 compressions to 2 ventilations results in less than 60% of the time devoted to chest compressions during resuscitation. Based on these observational data, we plan to retrain the first-responder system in June-July 2004 to use 30 compressions: 2 ventilations. At the same time, AEDs will be reprogrammed to allow more hands-on time during chest compressions. This activity will be quality improvement, although we plan to observe and collect data as part of our ongoing research database.

Support: Hypothermia and Gene Expression after Cardiac Arrest, (#R01 NS046073) National Institute of Neurological Disorders and Stroke (07/99 – 06/04) total award $848,032 ($166,250 direct costs + $80,631 indirect costs per year) Clifton W. Callaway, MD, PhD, PI. Vasopressin in Cardiac Arrest, Pittsburgh Emergency Medicine Foundation, total award $1525, Clifton W. Callaway, MD, PhD, PI.

B. Pediatric Cardiopulmonary Resuscitation

There is an expanding pediatric cardiac arrest program at the Safar Center that now has both bench and clinical components. Dr. Robert Clark (see prior report in TBI), Associate Professor in the Department of Critical Care Medicine, Associate Director of the Safar Center and Pediatric Critical Care Medicine specialist at Children's Hospital, has received funding from Children's Hospital of Pittsburgh to initiate laboratory studies in a new model of asphyxial cardiopulmonary arrest in rats. This research is off to a spectacular start. In addition, Dr. Robert Hickey in the Department of Pediatrics, Division of Emergency Medicine at Children's Hospital of Pittsburgh has ongoing mechanistic studies in the area of developmental brain injury and has played a key role in the national guidelines committees in resuscitation. Finally, Dr. Howard Ferimer of the Mercy Hospital Department of Pediatrics completed some studies in asphyxial cardiopulmonary arrest in collaboration with Dr. Edwin Jackson.

1. Laboratory Research in Pediatric Resuscitation

A. Asphyxial Cardiopulmonary Arrest in the Developing Rat (Robert Clark, MD)

In 1995, Drs. Larry Katz and Peter Safar published a seminal paper in the *Journal of Cerebral Blood Flow and Metabolism* describing a clinically relevant model of asphyxial cardiopulmonary arrest in adult rats. Based on that work, and with special talents of senior laboratory technician, Henry Alexander, Dr. Robert Clark developed an important pediatric analog of that asphyxial cardiopulmonary arrest model using post-natal-day (PND) 17 rats. This is an important development in that the PND 17 rat models a toddler or young child—the population most commonly afflicted by cardiopulmonary arrests resulting from asphyxiation (i.e., near drowning, trauma, child abuse, choking, SIDS). Although there are established models of perinatal ischemia, there are no small animal models mimicking cardiopulmonary arrest. Equally important is the fact that this is a

clinically relevant model that includes a global insult to the entire organism and all of the standard clinical components of resuscitation as guided by contemporary pediatric advanced life support (i.e., mechanical ventilation, chest compressions, epinephrine).

This year, T32 fellow Dr. Ericka Fink published the description of the model as a full manuscript in the journal *Pediatric Critical Care Medicine*. In addition, Dr. Fink presented three abstracts of her work in this exciting new model. She presented abstracts, at both the Society for Neuroscience and the Fellows' Research Day Conferences held by the Pennsylvania/Delaware Affiliate of the American Heart Association, reporting beneficial effects of post-resuscitation mild hypothermia on histopathological and functional outcome in this model. She also presented studies demonstrating a gender-dependent effect on behavioral outcomes and rate of neuronal death in this model. These findings lend further support to the notion that male neurons are more apt to die of necrosis while female neurons tend to die in a more delayed fashion—presumably via apoptotic mechanisms. That paper was presented at the 2004 meeting of the Society for Pediatric Research. Dr. Fink has been extremely productive and has taken great advantage of this new model. She is preparing a manuscript on the hypothermia work and is beginning studies of novel antioxidants as therapeutics in this model. Finally, Dr. Clark has submitted an RO-1 award on this model, and we are pleased to say that funding of this new program is anticipated.

Support: Children's Hospital of Pittsburgh. Robert SB Clark, MD, PI. The Laerdal Foundation for Acute Medicine. Ericka Fink, MD, PI

B. Developmental Aspects of COX-2-mediated Brain Injury (Robert Hickey, MD)

Dr. Robert Hickey continued work on his KO-8 award from NICHD to study developmental aspects of the role of COX-2 in brain injury. This research is being carried out under the mentorship of Dr. Steven Graham in the department of Neurology at the VA Hospital. Dr. Kochanek is a co-sponsor of the grant. COX-2 plays an important role in secondary injury in models of stroke, trauma, and cardiac arrest in adult investigation. Its role in pediatric brain injury remains to be defined. Studies to evaluate the effects of COX-2 inhibitors in the developing rat subjected to asphyxial cardiopulmonary arrest are in progress. Dr. Hickey also began work in neuronal culture to study potential mechanisms whereby COX-2 can contribute to ischemic injury.

Support: COX-2 and Injury in the Immature Brain, KO-8 (#HD40848) National Institute of Health, National Institute of Child Health and Development, (7/01-7/06), total award $623,430 ($115,450 direct + $9,236 indirect per year), Robert W. Hickey, MD, PI, Steven Graham, MD, Patrick Kochanek, MD, Co-Investigators; Robert Clark, MD, C. Edward Dixon, PhD, Peter Safar, MD, Consultants. COX-2 and Excitotoxicity in Developing Rat Brain, Competitive Medical Research Fund (CMRF), University of Pittsburgh, (7/1/03-6/30/05), total award $25,000. Robert W. Hickey MD, PI: Steven H Graham MD, PhD, Co-investigator.

Public Education and National Guidelines Committee

Dr. Robert Hickey is the current Vice-Chair of the American Heart Association Emergency Cardiovascular Care Committee (ECC) and the immediate past-chair of the American Heart Association subcommittee on Pediatric Resuscitation. The ECC is responsible for overseeing the American Heart Association's pediatric advanced life support (PALS), advanced cardiovascular life support (ACLS) and basic life support (BLS) courses. The AHA has approximately 250,000 instructors that train over 7 million people annually. Dr Hickey developed a new teaching module for the PALS course entitled "Catastrophic Illnesses in Children Presenting with Common Chief Complaints". In his capacity as Vice-Chair of the ECC, Dr. Hickey also serves as a representative to the International Liaison Committee on Resuscitation (ILCOR) and has recently participated in meetings in Australia, Italy, Brazil, and Budapest to develop international consensus on new developments in resuscitation science. Dr. Hickey is the Co-Chair of the ILCOR Pediatric Taskforce responsible for developing the evidence-based Pediatric Guidelines scheduled for release in 2005. Dr. Hickey also serves on the Science Advisory and Coordinating Committee (SACC) of the AHA. SACC advises the AHA on scientific issues and helps to develop the strategic goals and research initiatives of the AHA.

Pediatric Cardiopulmonary Arrest: Clinical Studies

Dr. Hickey has initiated the assembly of a multidisciplinary team to evaluate children resuscitated from cardiac arrest. The team has representatives from the entire continuum of care including pre-hospital, emergency medicine, critical care, neurology, neuroimaging, behavioral pediatrics, and rehabilitation medicine. The team will, 1) characterize early molecular markers of hypoxic ischemia brain injury, 2) evaluate strategies for prognosis of neurologic recovery, 3) identify patterns of functional deficits in long-term survivors, and 4) develop targeted strategies for rehabilitation of patients with hypoxic ischemia brain injuries. This information will facilitate comprehensive evaluation and treatment for individuals suffering from hypoxic ischemia brain injury and also develop a profile of the natural history of injury and recovery that can be used for evaluation of anticipated neuroprotective therapies. The study has been approved by the IRB and enrollment has begun.

Peer-Reviewed Manuscripts: Cardiopulmonary Arrest Program

1. Du L, Bayır H, Lai Y, Zhang Z, Kochanek PM, Watkins SC, Graham SH, Clark RSB: Innate gender-based proclivity in response to cytotoxicity and programmed cell death pathway. J Biol Chem (in press).

2. Fink EL, Alexander H, Marco CD, Dixon CE, Kochanek PM, Jenkins LW, Lai YC, Donovan HA, Hickey RW, Clark RSB: Experimental model of pediatric asphyxial cardiopulmonary arrest in rats. Pediatr Crit Care Med 5:139-144, 2004.

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4. Lai CS, Hostler D, D'Cruz BJ, Callaway CW: Prevalence of troponin-T elevation during out-of-hospital cardiac arrest. Am J Cardiol 93:754-756, 2004.

5. Sherman LD, Flagg A, Callaway CW, Menegazzi JJ, Hsieh M: Angular velocity: a new method to improve prediction of ventricular fibrillation duration. Resuscitation 60:79-90, 2004.

6. Menegazzi JJ, Callaway CW, Sherman LD, Hostler DP, Wang HE, Fertig KC, Logue ES: Ventricular fibrillation scaling exponent can guide timing of defibrillation and other therapies. Circulation 109:926-931, 2004.

7. Menegazzi JJ, Wang HE, Lightfoot CB, Fertig KC, Chengelis NL, Sherman LD, Callaway CW: Immediate defibrillation versus interventions first in a swine model of prolonged ventricular fibrillation. Resuscitation 59:261-270, 2003.

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Chapters, Editorials and Invited Papers: Cardiopulmonary Arrest Program

1. Clark RS, Lai Y, Hickey RW: Hypoxic-Ischemic Encephalopathy: Pathobiology and Therapy of the Post-Resuscitation Syndrome in Children. In: Pediatric Critical Care 3rd edition, Fuhrman B, Zimmerman J (eds.), Elsevier Science, St. Louis (in press).

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5. Kochanek PM: World Congress on Drowning, 2002: Task- Force on "Brain Protection" Pediatric Considerations (in press).

6. Kochanek PM, Hickey RW, Bayır H, Fink EL, Ruppel RA, Clark RSB: Pediatric neurointensive care. In: Textbook of Critical Care 5th Edition, Fink MP, Abraham E, Kochanek PM, Vincent JL (eds.), WB Saunders, Philadelphia, Chapter 60 (in press).

7. DeFranco DB, Ho L, Falke E, Callaway CW: Small molecule activators of the heat shock response and neuroprotection from stroke. Current Atherosclerosis Reports 6:295-300, 2004.

Abstracts: Cardiopulmonary Arrest Program

1. Fink E, Marco CD, Donovan HA, Alexander H, Dixon CE, Kochanek PM, Jenkins LW, Clark RS: Brief induced hypothermia improves outcome in a pediatric model of asphyxial cardiopulmonary arrest in rats. Crit Care Med 31:89, 2003.

2. Fink EL, Alexander H, Donovan H, Marco C, Dixon CE, Kochanek PM, Clark RSB: Therapeutic hypothermia improves neurologic recovery in a model of pediatric asphyxial cardiopulmonary arrest in rats. Society for Neuroscience 2003 (electronic).

3. Fink EL, Marco CD, Donovan HA, Alexander H, Dixon CE, Kochanek PM, Jenkins LW, Clark RS: Brief induced hypothermia improves outcome in a pediatric model of asphyxial cardiopulmonary arrest in rats. American Heart Association; Pennsylvania/Delaware Affiliate Fellows' Research Day, Hilton Hotel, Pittsburgh, PA, February 13, 2004.

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8. Ramos R, Menegazzi J, Wang H, Callaway C: Post-resuscitation hemodynamics and relationship to duration of ventricular fibrillation. Prehosp Emerg Care 8:81, 2004.

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SHOCK AND SUSPENDED ANIMATION PROGRAM

The hemorrhagic shock (HS) and suspended animation (SA) program consists of project I on HS in rats and pigs (PI, Dr. Tisherman; Co-PI, Dr. Safar); and project II on suspended animation (SA) in dogs (PIs, Dr. Safar [7/03] Dr. Kochanek [8/03-6/04]; Co-PI, Dr. Tisherman). The funding since 1997 was made possible through special "plus-up" funds from Congress initiated by former Navy Commander Lyn Yaffe, MD during 2001/2003. The HS studies were funded separately by the Office of Naval Research. During the 2003/2004 academic year, separate funding was completed on the rat HS program, and both of these programs were supported by our re-tooled and expanded single program entitled "Emergency Hypothermia" again funded by Congress through Telemedicine and Advanced Technology Research Center (TATRC). We received total funds of approximately $956,949 during 2003/2004.

Our research ICU for large animals, initiated in the 1970s, is still considered a unique resource for the documentation of novel cardiopulmonary cerebral resuscitation methods. It must be maintained continuously to be cost-effective, with at least three technicians, two full-time MD research fellows with CCM experience, and about 40 long-term large animal experiments per year. Maintaining this ICU program alone requires over $0.5M per year. In 2003/2004, the research fellows were Dr. Ala Nozari (in his third year) and Dr. Xianren Wu (in his fifth year); Mr. William Stezoski has continued as lab coordinator. This was the late Dr. Safar's final year as PI of this important project. Subsequent to his passing, Dr. Kochanek assumed the role of PI of this project with Dr. Tisherman as Co-PI. Drs. Kochanek and Tisherman will work together on this important project. Specifically, Dr. Kochanek will serve as overall PI and PI for the laboratory work, with Dr. Tisherman as Co-PI. While, for the planned clinical feasibility trial of SA in the future, Dr. Tisherman will serve as PI. Co-investigators or consultants included Drs. Yaffe, Klain, Jackson, Dixon, Clark, Kagan, Jenkins, and Radovsky, and most recently Robert Wagner (DVM), Joseph Carcillo, and Robert Garman.

The objective of the HS-SA program has been to help maximize the reversibility of presently lethal traumatic hemorrhage resulting in exsanguination CA (ExCA). The HS studies in rats and pigs were designed to extend the golden hour of HS tolerance; HS (low blood flow), with viscera as the most vulnerable organs, is the prevalent cause of death in soldiers "dying of wounds" (DOW). Exsanguination cardiac arrest (ExCA) (no blood flow), with the brain as the most vulnerable organ, is the prevalent cause of death in soldiers "killed in action" (KIA). SA is a totally new approach for presently unresuscitable conditions. While SA has been considered science fiction, colleagues are now increasingly using this term seriously, as representing rapidly induced preservation of the organism for delayed resuscitation. This idea was initiated in the 1980s by Drs. Safar and COL Ronald Bellamy. For HS and SA we have explored mainly hypothermic strategies – specifically mild hypothermia (33-36°C) for HS and profound hypothermia (5-15°C) for SA. In the laboratory, we plan to extend the duration of SA to address the practicalities of prolonged transport. In addition, we plan to develop a SA model in rats to take advantage of the many molecular tools available for use in rats that are not

available for use in dogs or pigs. Finally, Dr. Tisherman, as discussed above, is planning clinical feasibility studies for both in selected trauma centers. Devices needed for such studies are being developed concurrently with additional laboratory studies, including collaboration with our industrial partners (Ardiem Medical for cooling devices, and Dr. Yaffe's group working on smart catheters and other aspects of trauma bay and field application of this exciting technology.

The HS models in rats and SA models in dogs used in 2002/2003 had been initiated and further developed over the years by our group. They have several unique features, the most important being clinical relevance in terms of insult, resuscitation strategy, ICU management, and outcome.

1. HS Studies

Work in the HS program during academic year 2003/2004 included publication of studies that were completed last year from work funded by the Office of Naval Research (PI: SA Tisherman, MD; Co-PI: P Safar, MD), and studies supported by the "Emergency Hypothermia" congressional appropriation. Fellow Xianren Wu, MD supervised all studies. The rat studies were completed by technician Jason Stezoski. The pig studies utilized the ICU team led by S. William Stezoski, with technicians Jeremy Henchir, Sherman Culver, Alan Abraham, Jason Stezoski, Scott Kostelnik, and Murugan Subramanian. Fellow Ala Nozari, MD, also assisted with the pig studies.

Mild Hypothermia and Prolonged HS

As described in last year's report, we showed, using models of uncontrolled HS or pressure-controlled HS, that a mean arterial pressure (MAP) of 50 mmHg was insufficient to allow long-term survival after very prolonged (6 h) HS. Even a MAP of 60 did not consistently allow survival. We also showed that mild hypothermia (34°C) improved survival after prolonged HS. A full manuscript is in preparation.

Mild Hypothermia and HS: Mechanisms of Benefit

This year, Dr. Wu published two full manuscripts in this area from work previously completed. First, he studied a variety of mechanistic endpoints to probe the potential mechanisms underlying the beneficial effects of mild hypothermia in HS. Mild hypothermia conferred protection to the liver but did not attenuate increases in either cytokines or markers of oxidative stress. Early increases in blood glucose, and reductions in both serum potassium and transaminases were seen with hypothermia. That paper was published in the *Journal of Trauma*. In a second paper, Dr. Wu demonstrated that after spontaneous hypothermia during HS, continued mild hypothermia did not improve long term outcome, but favorably influenced survival time, particularly with severe HS. Both of these studies suggest that the beneficial effect of mild hypothermia in HS is early, and attenuation of late secondary injury mechanisms by hypothermia is not readily seen. This paper was also published in the *Journal of Trauma*.

Solutions and HS

Recent fellow graduate Dr. Rainer Kenter carried out several studies (described in prior reports) examining the optimal fluid for hypotensive (limited) resuscitation during HS. This includes work evaluating hypertonic and hyperoncotic solutions and related combinations. Full manuscripts of that work are being prepared by Dr. Kentner.

Hypothermia and Poikilothermia

In last year's report, we discussed the preliminary work of Dr. Wu testing the new neurotensin analog (NT-69L), which former fellow Dr. Larry Katz found to induce rapid and sustained mild hypothermia in rats after asphyxial CA. As discussed, a poikilothermic state could be extremely essential to the benefits of mild hypothermia, and a pharmacological agent that induces hypothermia is an appealing concept. However, in our volume-controlled HS model, NT had no further beneficial effect on survival than did our standard application of hypothermia via surface cooling. This highlights the fact that one must carefully determine the optimal application conditions for hypothermia (target temperature, duration, re-warming rate, sedation/analgesia, and associated pharmacological agents) in each disorder that it used. This work is in preparation by Dr. Wu as a full manuscript.

Large Animal Outcome with Mild Hypothermia

Previous studies of mild hypothermia during HS have been performed in rats. Clinically, there is great concern that hypothermia is associated with worse outcomes in trauma patients. Prior to making final plans for clinical trials of mild hypothermia during HS in trauma patients, we felt that a large animal study using a clinically-relevant model of HS plus trauma, with prolonged life support, was needed. Also, we wanted to test the safety and efficacy of ice-cold fluid infusion for induction of hypothermia during HS. Studies have suggested benefit in patients after CA. Thus, we developed a pig HS model with controlled continuous bleeding (75 ml/kg over 3 h) and trauma induced by laparotomy and splenic transection (delayed splenectomy). At HS 40 min (simulating arrival of paramedics) pigs were randomized into 3 groups: Group-1, normothermia (38°C) with warmed saline, Group-2, hypothermia (34°C) induced with 2°C i.v. saline and surface cooling, and Group-3, hypothermia (34°C) with 24°C i.v. saline and surface cooling. Resuscitation fluids were given when MAP was <30 mmHg until HS 3 h. Remarkably, rapid cooling with ice-cold saline was not as effective as slower i.v. cooling using room temperature resuscitation fluids. Use of ice-cold fluid increased blood pressure and lactate. It may be that rapid cooling using ice-cold fluid during HS produces peripheral vasoconstriction, leading to underestimate of the fluid needs. Further studies are needed to prove that hypothesis. This year, Dr. Wu presented those interesting results at the Fellows' Research Day hosted by the American Heart Association, Pennsylvania/Delaware Affiliation.

In this area of research, Dr. Tisherman also published a review in the *Journal of Trauma* entitled Trauma Fluid Resuscitation 2010, and several related chapters in the Saunders Manual of Critical Care.

2. SA Program

Studies in Dogs

A. Successful SA after 2 hours of ExCA

In prior reports, we described studies by former fellow Wilhelm Behringer in our group demonstrating that an SA of 2 hours in duration could be achieved with intact outcome in some animals. Intact outcome for SA of 90 minutes in duration was remarkably consistent using a target tympanic temperature of 10°C. These landmark studies were published this year as a full paper in the journal *Critical Care Medicine*.

B. Addition of Tissue Trauma to the SA Model and the Effects of Plasma Exchange

In prior SA experiments, we reported that induction of profound cerebral hypothermia (a tympanic membrane temperature of ~10°C) can allow intact survival after a 90 min ExCA. The potential benefits of drugs or specialized solutions have been disappointing. The anti-oxidant Tempol and a specially developed fluid (by Michael Taylor, PhD) for organ preservation with hypothermia (Unisol) seem promising. In last year's report, our studies suggested that the addition of tissue trauma (thoracic incision, laparotomy, splenectomy) caused extracerebral organ system dysfunction, although brain histopathology is normal after 60 min SA. This year, Safar Center Fellow Dr. Ala Nozari published the results of that study in the *Journal of Trauma*.

In children with multiple organ dysfunction and thrombotic microangiopathic anemia, use of plasma exchange has resulted in significant clinical improvement. Thus, we hypothesized that plasma exchange might help alleviate some of the extracerebral complications seen after trauma and SA. After 120 min SA in dogs, plasma exchange decreased the coagulopathy and improved overall performance, without affecting neurologic deficits and brain histopathology. These studies support the potential use of 2 h of SA even in the setting of ExCA that is accompanied by considerable tissue trauma. This is an important study toward the potential clinical use of SA. Plasma exchange may be needed as a clinical adjunct. However, it must be recognized that our studies in dogs have been carried out without the resources of a canine blood bank (i.e., we are limited by lack of therapies such as platelets, cryoprecipitate, and fresh-frozen plasma). The results of this interesting study were presented at the 2003 meeting of the Society of Critical Care Medicine. These studies were carried out in collaboration with Ann Hale of Midwest Laboratories who provided blood typing for the plasma exchange. We are grateful to Ann for her help with these complex studies.

C. Small Volume Induction of SA using Veno-Arterial Re-circulation

SA in its current form could be applied in a trauma bay or operating room in the setting of civilian or military trauma. However, one of the greatest potential limitations to the application SA—in its current form—in the field is the need for large (20 liters or more) quantities of iced flush solution. Pharmacologic adjuncts to the flush solution are one possibility. In the laboratory, another approach that can be used is to re-circulate the flush solution. This year, Dr. Nozai successfully tested this approach in our dog model using a

90 min SA protocol. This approach, using a femoral route for delivery, proved to be highly efficacious. These findings were presented at the 2003 congress of the Society of Critical Care Medicine. Recirculation of the flush is an interesting potential approach for lab use—specifically in models without tissue trauma. However, wounds with vascular disruption—and resultant loss of the flush solution rather than re-circulation—would prevent the use of this approach for clinical ExCA. Re-circulation, however, could be used for normovolemic CA—in situations where SA might be considered, such as refractory CA.

D. Mild Hypothermia During CPCR Rather Than after Resuscitation

Recent clinical trials have demonstrated beneficial effects of mild hypothermia in adults when induced by surface cooling after restoration of spontaneous circulation (ROSC). This year, Dr. Ala Nozari was the team leader on an important study at our center that tested the hypothesis that benefits of mild hypothermia would be even greater if it were begun during CPCR. The most likely scenario for benefit of this approach would be the prolonged resuscitation of a patient refractor to ACLS. To test this hypothesis, we used extracorporeal veno-venous cooling during prolonged (a total 40 min insult including 3 minutes of no-flow, 7 minutes of BLS, and 30 min of ACLS) CPCR to achieve target temperatures of either normothermia mild (34°C) or moderate (27°C) hypothermia. With this approach mild or moderate hypothermia during CPCR dramatically improved outcome at 96 hours after resuscitation. This work was published this year as a full paper, published by Dr. Nozari in *Critical Care Medicine* and strongly suggests the need to carry out clinical trials of intra-arrest cooling. This manuscript was the subject of a very favorable editorial.

We are beginning several new projects using the dog SA model, including pilot work to study the impact of prolonged HS prior to SA and preliminary investigation to test approaches to break the two-hour barrier of SA. New additional facets of our work that we also hope will further our goals will be the addition of neuropathologist Dr. Robert Garman to our group and expanding our collaboration with Dr. Carleton Hsia and his talented group at Synzyme. More to follow on these important new approaches and collaborations in next year's report.

Studies in rats

A. Proteomic Studies in a Rat model of Complete Global Cerebral Ischemia Without Reperfusion

Two years ago, Drs. Larry Jenkins and Peter Safar initiated a project linked to the SA program that seeks to probe into the mechanisms of cellular (neuronal) degradation at prolonged global cerebral ischemia during profound hypothermia—at levels of cooling that are successfully used in our SA experiments. An intriguing question is–during prolonged (1-2 h or more) complete global cerebral ischemia (at profound hypothermia, 10°C), what cellular derangements occur. Is cellular degradation during prolonged periods of hypothermia occurring, does it set the stage for damage during reperfusion, or is reperfusion and re-warming the key? Although mechanisms involving lipid degradation, or DNA or RNA damage, may be important, a key initial focus of our work

in this area has been on proteolytic damage. Dr. Jenkins has used proteomics to study protein degradation and post-translational modification in TBI and previously published a manuscript on this approach. In last year's annual report, the initial work on this project was described including work by Dr. Jenkins, and PICU T-32 fellow Dr. Mandeep Chadha, who used 2-D gel electrophoresis to examine the effect of 30 min of complete global brain ischemia (at either normothermia or profound hypothermia, 10°C) on the proteome of the isolated rat hippocampus. Their initial work suggested that 30 min of complete global brain ischemia produced only modest changes in the proteome of the rat hippocampus. Obviously, the limitations of the sensitivity of 2-D gel electrophoresis for low copy proteins and protein fragments must be taken into consideration. Nevertheless, these initial studies are provocative. This work was presented by Dr. Chadha at both the NCMRR/NIH Training Workshop and at the American Heart Association Pennsylvania/Delaware Affiliate Fellows' Research Day. Dr. Chadha was selected as having one of the three top presentations at the NCMRR/NIH conference—congratulations. Studies of the impact of longer ischemia durations and the effect of reperfusion are underway using the rat SA model descried below.

B. A Rat Model of SA

In the middle of this academic year, Dr. Tomas Drabek—a cardiac anesthesiologist from Prague—joined our group. His specific goal is to develop a rat model of SA that can be used both for molecular studies and to screen therapies. Drs. Drabek, Kochanek and technician Jason Stezoski visited the laboratories of Drs. David Warner and Hillary Grocott to acquire the method of cardiopulmonary bypass in rats which is critical to the resuscitation phase of SA—and thus to the development of a rat SA model. We wish to thank them for their remarkable help and generosity and Dr. Drabek and Jason Stezoski have begun to carry out the initial studies in this important and novel direction for our laboratory.

C. Device Development

During the 2003/2004 academic year, Safar Center investigators (Drs. Safar, Tisherman, and Kochanek, and Mr. William Stezoski) working on the SA project continued to provide consultation to Dr. Lyn Yaffe and his "Smart Catheter" group working on the development of novel catheters for the clinical and experimental implementation of SA. We continue to evaluate catheter prototypes for aortic insertion. In addition, this same group of Safar Center investigators provided consultation to the Ardiem Medical Company in the development of cooling devices for use in induction of hypothermia, both in SA and HS paradigms. We continue to meet with the development team of Ardiem Medical at the Safar Center and are currently using their initial devices for experimental cooling in our SA and mild hypothermia projects in large animals.

D. Emergency Hypothermia, Clinical Planning

Dr. Tisherman is beginning to organize an initial meeting of a clinical consortium for SA—including important and interested trauma centers across the USA. As we look to

the future, our new congressional application will be entitled "Applied Emergency Hypothermia," to highlight the fact that we feel that we are approaching the possibility of a clinical feasibility trial. This will build on the initial groundwork laid by Dr. Tisherman with investigators in the American Association for the Surgery of Trauma (see last year's report for details). Future meetings of this consortium group of potential investigators are planned.

Finally, several review articles on SA were published this year by Drs. Tisherman, Yaffe and/or Kochanek. Most notable are the outstanding review articles on SA and "Smart Catheter" approaches, written by Drs. Tisherman and Yaffe, respectively that were published in the Festschrift to Dr. Safar published in the journal *Critical Care Medicine*. Dr. Kochanek was the lead author of a chapter on SA in the new textbook on hypothermia published this year by Dr. Hayashi.

Support: Novel Resuscitation from Lethal Hemorrhage. Suspended Animation for Delayed Resuscitation, US Army-Combat Casualty Care, DAMD 17-0102-0038 (9/15/03-9/14/04), $956,949, Patrick M. Kochanek, MD, PI and Samuel Tisherman, MD, Co-PI

Peer-reviewed Manuscripts: Shock and Suspended Animation Program

1. Behringer W, Safar P, Wu X, Kentner R, Radovsky A, Kochanek PM, Dixon CE, Tisherman SA: Survival without brain damage after clinical death of 60-120 min in dogs using suspended animation by profound hypothermia. Crit Care Med 31:1523-1531, 2003.

2. Nozari A, Safar P, Stezoski SW, Wu X, Henchir J, Radovsky A, Hanson K, Klein E, Kochanek PM, Tisherman S: Mild hypothermia during prolonged cardiopulmonary-cerebral resuscitation increases conscious survival in dogs. Crit Care Med (in press).

3. Nozari A, Safar P, Wu X, Stezoski WS, Henchir J, Kochanek PM, Klain M, Radovsky A, Tisherman: Suspended animation can allow survival without brain damage after traumatic exsanguination cardiac arrest of 60 min in dogs. J Trauma (In press).

4. Tisherman SA, Barie P, Bokhari F, Bonadies J, Daley B, Diebel L, Eachempati SR, Kurek S, Luchette F, Puyana JC, Schreiber M, Simon R: Clinical practice guideline: Endpoints of resuscitation. J Trauma (in press).

5. Wu X, Stezoski J, Safar P, Bauer A, Tuerler A, Schwarz, N, Kentner R, Behringer W, Kochanek PM, Tisherman SA: Mild hypothermia during hemorrhagic shock in rats improves survival without significant effects on inflammatory responses. Crit Care Med 31:195-202, 2003.

6. Wu X, Stezoski J, Safar P, Nozari A, Tisherman SA: After spontaneous hypothermia during hemorrhagic shock (HS), continuing mild hypothermia (34°C) improves early, but not late, survival in rats. J Trauma 55:308-316, 2003.

Chapters, Monographs, and Editorials: Shock and Suspended Animation Program

1. Kochanek P, Tisherman S, Stezoski SW, Nozari A, Wu X, Safar P: Novel potentials for emergency hypothermia: suspended animation with delayed resuscitation from exsanguinations cardiac arrest. In: Brain Hypothermia 2004, Hayashi (ed.), Springer-Verlag Publishers (in press).

2. Tisherman SA: Trauma fluid resuscitation 2010. J Trauma 54:231-234, 2003.

3. Tisherman SA: Suspended animation for resuscitation from exsanguinating hemorrhage. Crit Care Med 32:S46-50, 2004.

4. Tisherman SA: Myocardial contusion. In: Saunders Manual of Critical Care. Kruse JA, Fink MP, Carlson RW (eds.), Saunders, Philadelphia, 2003, pp 73-74.

5. Tisherman SA: Abdominal trauma. In: Saunders Manual of Critical Care. Kruse JA, Fink MP, Carlson RW (eds.), Saunders, Philadelphia, 2003, pp 491-493.

6. Tisherman SA: Abdominal aortic aneurysms. In: Saunders Manual of Critical Care. Kruse JA, Fink MP, Carlson RW (eds.), Saunders, Philadelphia, 2003, pp 509-511.

7. Tisherman SA: Tertiary survey of the trauma patient. In: Trauma: Resuscitation, Anesthesia, & Critical Care. Wilson WC, Grande CM, Hoyt DB (eds.), Marcel Dekker, Inc. (in press).

Abstracts: Shock and Suspended Animation Program

1. Chadha MS, Peters G, Zhang X, Safar P, Kochanek PM, Jenkins LW: The effects of hypothermia on rat hippocampal proteomic profiles after 30 minutes of complete cerebral ischemia. National Center for Medical Rehabilitation Research, National Institute of Child Health and Human Development, NIH and National Institute of Neurological Disorders and Stroke Training Workshop, Bethesda, MD, December 9-10, 2003.

2. Chadha MS, Peters G, Zhang X, Safar P, Kochanek PM, Jenkins LW: The effects of hypothermia on rat hippocampal proteomic profiles after 30 minutes of complete cerebral ischemia. American Heart Association; Pennsylvania/Delaware Affiliate Fellows' Research Day, Hilton Hotel, Pittsburgh, PA, February 13, 2004.

3. Nozari A, Safar P, Stezoski SW, Wu X, Kochanek PM, Henchir J, Culver S, Tisherman S: Suspended animation for 90 min cardiac arrest (CA) in dogs with small volume arterial flush and veno-arterial extracorporeal cooling. Crit Care Med 31:A9, 2003.

4. Nozari A, Safar P, Tisherman S, Stezoski S, Kochanek P, Wu X, Kostelnik S, Carcillo J: Suspended animation and plasma exchange enables full neurologic recovery from lethal traumatic exsanguinations, even after 2h period of no flow. Crit Care Med 31:A9, 2003.

5. Nozari A, Safar P, Wu X, Stezoski WS, Henchir J, Kochanek P, Klain M, Radovsky A, Tisherman SA: Suspended animation can allow survival without brain damage after traumatic exsanguination cardiac arrest of 60 min in dogs. J Trauma (in press).

6. Nozari A, Safar P, Stezoski SW, Wu X, Henchir J, Radovsky A, Hanson K, Klein

E, Kochanek PM, Tisherman S: Mild hypothermia during prolonged cardiopulmonary-cerebral resuscitation increases conscious survival in dogs. Critical Care Medicine (in press).

7. Tisherman S. Suspended animation for resuscitation from exsanguinating hemorrhage. Crit Care Med 32(2 Suppl):S46-50, 2004.

8. Wang HE, Callaway CW, Peitzman AB, Tisherman SA: Admission hypothermia is associated with adverse outcomes after trauma. Acad Emerg Med 11:513-514, 2004.

9. Wu X, Stezoski J, Safar P, Nozari A, Kochanek P, Tisherman S, Richelson E: Compared to controlled normothermia, spontaneous hypothermia, with or without neurotensin, improves survival during hemorrhagic shock in awake rats. Crit Care Med 31:A29, 2003.

10. Wu X, Kochanek PM, Tisherman S: Mild Hypothermia Improves Survival after Prolonged Hemorrhagic Shock in Pigs. American Heart Association: Pennsylvania/Delaware Affiliate Fellows' Research Day, Hilton Hotel, Pittsburgh, PA, February 13, 2004.

Founding Director – Peter J. Safar, MD
1979 - 1994

Dr. Peter J. Safar received his MD degree in 1948 from the University of Vienna. He came to the United States permanently in 1954 along with his wife, Eva Kyzivat Safar. Over the course of his career Dr. Safar earned many awards and honors. He is generally considered the father of "CPR" and pioneered the development and implementation of this vital life-saving technique used worldwide. Dr. Safar also had important roles in the development of a number of other areas in medicine, such as the fields of intensive care and emergency medicine. He trained hundreds of anesthesiology, critical care medicine and emergency medicine specialists working around the globe. You can read about his many accomplishments and view his extensive publication list on the Safar Center website. Dr. Peter Safar was also highly revered by his colleagues, peers, and trainees.

In 1979, Dr. Safar founded the International Resuscitation Research Center (IRRC) after a highly successful tenure as the founding Chairman of the Department of Anesthesiology and Critical Care Medicine, 1961-1979. In July 1994, Dr. Safar turned over the directorship of the IRRC to Dr. Patrick M. Kochanek. One of Dr. Kochanek's first directives was the renaming of the IRRC to "Safar Center for Resuscitation Research."

On August 3, 2003, after a 15-month fight against cancer, the Safar Center for Resuscitation Research lost its Founding Director, a great colleague, and a loyal friend – Peter J. Safar, MD. Now that we can no longer walk into his office and ask for his advice, we must accept the challenge to continue his work and preserve his legacy for future generations of researchers and scientists. Not only was Dr. Safar our leader, mentor, and colleague for many years, but he was also a personal friend to many of us. Though he is greatly missed, the way he lived life to its fullest continues to inspire us to seek breakthroughs and make the world a better place for all.

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